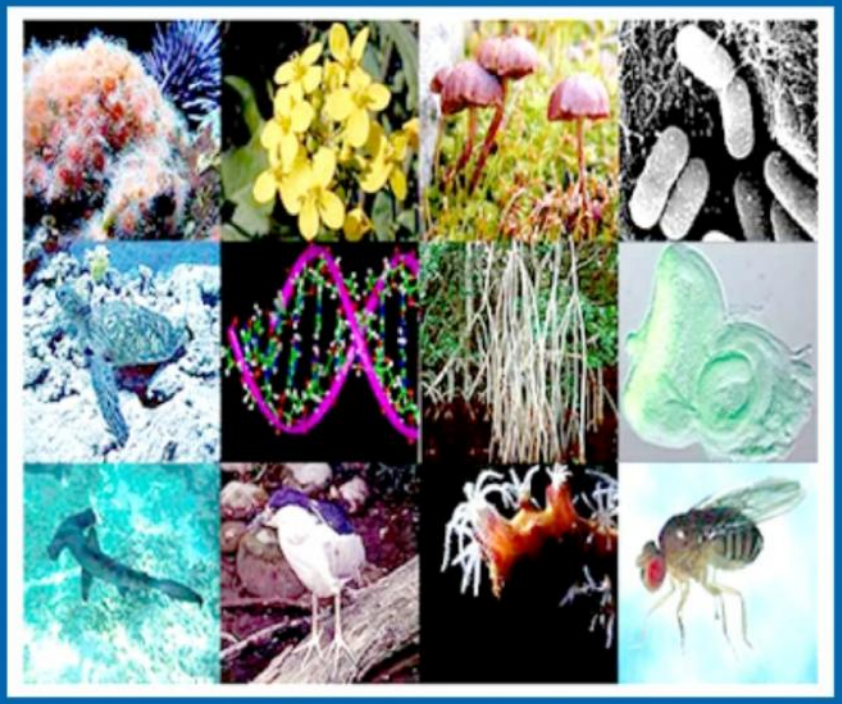

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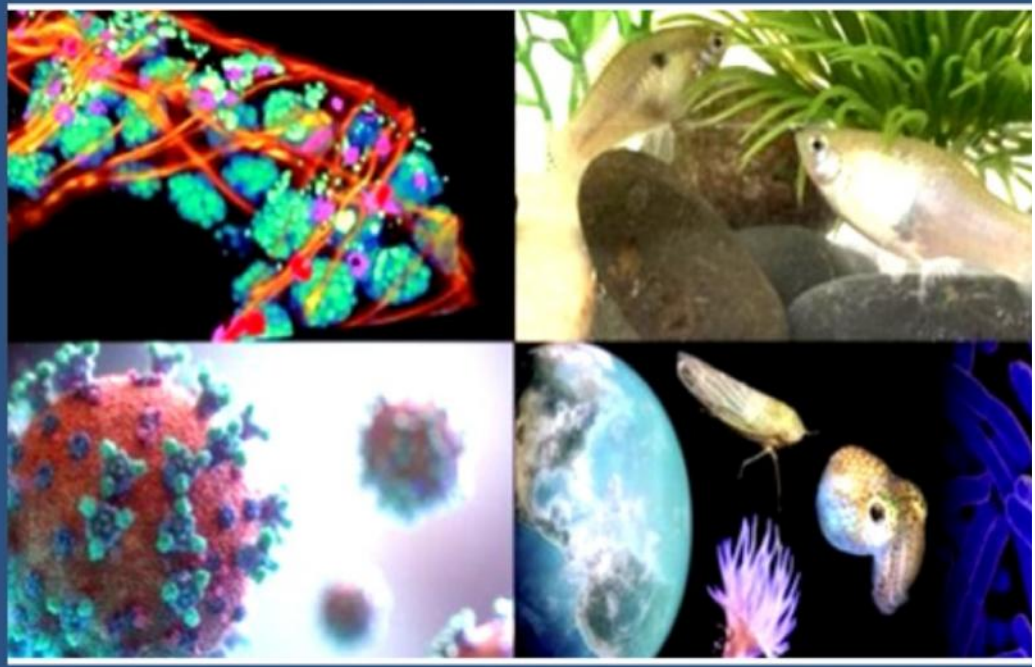
CURRENT ADVANCES IN BIOSCIENCES

First Edition

Editors
Dr. R. B. Tripathi
Dr. R. Deepa
Dr. A. Kiruthiga



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Preface

Current Advances in biosciences have brought about remarkable progress in various fields, including Pharmaceutical from animal, biochemistry, dental sciences, hydrology, space pollution, biotechnology, oral pathology, entomology,fishery,medical sciences, herbal medicines, food and nutrition, biodiversity, microbiology, gene and enzyme technology, metagenomics, organic farming, ecotechnology, nanotechnology and sustainable resource management in the context of fish, fisheries, and food. This book aims to provide an overview of the latest research and developments in these areas, shedding light on the intricate workings of nature and the implications for human health and ecological balance. This book presents a collection of recent research findings and updates across diverse fields in biosciences.It is our sincere hope that this compilation will inspire further research and foster collaborations to address the pressing issues facing our environment and human well-being

The book presents a comprehensive collection of chapters covering diverse topics in the medical and biological sciences. We aim to foster scientific curiosity, inspire further research, and contribute to the advancement of knowledge in these fields.

We are very much thankful to Thanuj International Publishers who readily accept and publish this subject. Also the author is very much thankful to Professor Indu Singh, Dr.Ashok Kumar, Subbulakshmi Packirisamy, D.E.Nirman Kanna, Dr.K.Padmavathy, Ms.A.Iswarya, T.Poornakala, T.Rajagopal, G.Archunan, P.Ponmanickam, Baiz Laika Riyaz, Saima Jan, Sumaira Rashid, Shafiya Mushtaq, K. Tamil Ilakkiya, Dr.Pavani Muddepalli, Ms.Jasmine Vinshia, Mr.Dinesh Kumar Thirugnanam, Dr.Manjusha Pouranik, Yogavathi P.S., Gayathri S., Dr.N.Aarthi, Ajay Kumar Shukla, Vimal Kumar Yadav, Vishnu Prasad Yadav, Aarti Tiwari, Pallavi Tiwari, Garima Verma, Manoj Kumar Mishra, Maya Sharma, Aruna K.M., Dr.K. Lakshmi Priya, Dr.K.Aishwarya, Dr.R.Riduvarshini, Dr.N.Ambalavanan, Dr.Karuna Kumari Jaddu, Dr.B.Ramakrishna, Dr.Sangeeta Sarkhel, K.Gautam, R.Sankar, J.Prasanna, S.Mahendran, P.Maheswari, M.Nivetha, Muniasamy, S.Gowtham, P.Ponmanickam, S.Ashok, Tania Sengupta Rathore, Sahana Singh, Sheenat Chawla, Humaira Jasmin, Sarika Kulkarni, Dr.G.V.Mali, Priya Sundararajan, Shanmuga Priya Ramasamy, Padmavathi M, Rooba Lakshmi S., Jennifer Princy R.,Sona A., PriyaSenanV, JyothilekshmiS, Divyasree A, Renuka Devi K.,Anuradha Jape, Devyani Salunke, Abhay Harsulkar, D., Jansirani, V.Ganapathi Gayathri, K.Muthulakshmi, K.Ajintha and Dr.J.Shifa Vanmathi for contributing their help and support for this work.

Editors

Dr. R. B. Tripathi
Dr. R. Deepa
Dr. A. Kiruthiga

About Editors

Dr.R.B.Tripathi is currently working as Assistant Professor in P.G.Department of Zoology, M.L.K.P.G.College, Balrampur-271201, Uttar Pradesh, India. He has been completed his Ph.D.in Zoology from Dr. R.M.L. Avadh University, Ayodhya, Uttar Pradesh, India. He has 22 years teaching experience in U.G and 18 years teaching experience in P.G classes, published 16 book chapters, 44 research papers in international and national reputed journals, participated and presented papers in many international and national seminars, conferences and workshops. He is Indian Zoologist, published by Surya Scientist Unique Researchers Yare Association, 2015. He is Associate Editor in International Journal of Advanced Research in Biological Sciences (ISSN:2348-8069), Editorial board member in International Journal of Advanced Multidisciplinary Research (ISSN: 2393-8870), 5 book Editor in Recent Trends in Life Sciences Research (ISBN: 978-81-947071-3-4), published by Darshan Publishers, Tamil Nadu, India, Recent Advancements and Research in Biological Sciences (ISBN:978-81-952529-1-6), Current Trends in Biological Sciences (ISBN:978-93-94638-00-6), Current Research in Life Sciences (ISBN: 978-93-94638-22-8) and Recent Research in Biosciences (ISBN:978-93-94638-25-9) published by Thanuj International Publishers Tamil Nadu,India.

Dr.R. Deepa M.Sc., M.Phil., Ph. D, currently working as an Associate Professor in the Department of Biochemistry, Madha Dental College and Hospital, Chennai. She has completed her M.Sc. Medical Biochemistry from Dr.A.L.M PG Institute of Basic Medical Sciences and M. Phil from A.C. Tech College, University of Madras, Chennai. She received her Ph.D. from Bharathiar University, Coimbatore and qualified for TNSET.She has 16 years of teaching experience in Biochemistry and guided students in preparing seminars and projects. She has published 19 research and review papers in peer reviewed national and international journals. She is the author of 9 book chapters and is a reviewer of Elsevier's book Nutrition for Nurses. Life member of many scientific societies and acted as a chairperson in national and international seminars and conferences.

Dr.A. Kiruthiga M.Sc., Ph. D, currently working as an Associate Professor in the Department of Microbiology, Madha Dental College and Hospital, Chennai. She has graduated with her B.Sc.Microbiology and secured University First Rank from the University of Madras in 2007. She specialized in M.Sc. Medical Microbiology (FACULTY OF MEDICINE) from Dr.A.L.M PG Institute of Basic Medical Sciences, University of Madras in 2010. She has been qualified in the Tamilnadu State Eligibility Test (TNSET) for Lectureship in the subject of Life Sciences in 2012. She received her Ph.D. from Bharath Institute of Higher Education and Research in 2022. She has actively participated in and presented posters and papers at various National and International Conferences. She has over 12 years of teaching experience in Microbiology for the students of BDS, MDS, B.Sc. Nursing, BPT and Allied Health Sciences. She has guided students in preparing seminars and ICMR projects. She has published 7 research and review papers in peer-reviewed national and international journals. She is a recipient of the Academic Excellence

Award and has authored 4 book chapters. She is a Life member of many scientific societies and acted as an organizing member and a chairperson in various National seminars and conferences. Presently she is actively involved in the fields of antibiotic resistance and molecular studies in Medical Microbiology.

Current Advances in Biosciences

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CONTENTS

Pharmaceutical from Animals

Dr. R.B. Tripathi
Assistant Professor
P.G. Department of Zoology, M.L.K.P.G.College,
Balrampur-271201(U.P.), India
E-mail: *drrbtripathi.77@gmail.com*

Introduction

The successes of modern medicines, derived from animals and animal products are due to collaborative research programmes of pharmaceutical industries and scientists of various fields. Before the world war 1st, the drugs were mostly prepared by synthetic chemicals but due to the cut-off in the supply of chemicals,the use of domestic animals for the pharmaceutical industry was started for this purpose. Since then and due to the new researches and developments of industrial technology it has gained good position in the field of medicine. The inventions of antibiotics and other anti-infective agents have certainly reduced the mortality due to number of contagious diseases which cause maximum death prior to adulthood.

The drugs prepared from the animals and animal products have good response in treatment without affecting the longevity of life. New drugs are being derived from the cardiac glycosides (digitoxin), protein hormones (insulin) and many others.The animals commonly used in the pharmaceutical industry are cats, guinea pigs, dogs, mice, rats and monkeys etc. It is thought that for the proper development of pharmaceutical industries a number of specialists including pharmacologists, pathologists, physiologists, endocrinologists, toxicologists, veterinarians, Pharmacists and medical advisers should work in collaboration with organic, analytical and physical chemists.

The industries for pharmaceutical purposes are highly specialized with regards to the application of biological science, chemistry and engineering to find out new useful drugs and make the drugs easily available to the medical persons and patients.

A: Immunization

The first introduction of specific antigen results in the production of a small amount of antibody which disappears from the blood plasma after some time. If a second dose of the same antigen is given after one month or more,the

response would be more.Now the cells of the body develop maximum amount of antibody to counteract the similar type of response in future.Thus,by the formation of antibody in advance of an infection,a number of diseases can be controlled.If proper eradication programme can be managed by the government,a number of diseases can be eradicated. Ex.-small pox. The vaccination is routinely used against a number of diseases viz. small pox, measles, diphtheria, tetanus and typhus fever and is gaining practical success. The principle of immunization has proved to be very much successful in eradicating a number of diseases.

B: Serum Theory

The use of antibodies for the treatment of diseases came in practice in 1890. The antitoxins prepared by the immunization of horses is being used successfully against diphtheria.The antiserum was used commonly to control the tetanus of the soldiers in World War 1st. But the use of antibody,synthesized in the horse,result in the formation of antibodies against the horse serum protein which causes 'serum sickness' in human beings.To avoid the serum sickness,the use of human antibodies has been introduced in medical science.Other diseases like rabies and hepatitis are also cured by the use of serum products. The thymus glands is the organ responsible for the immunity response of mammals. Cortisone obtained from adrenal glands of animals, is used for the cure of rheumatoid arthritis.

C: Animal Oriented Medicines

1:Leech (Genus-Hirudinaria,Phylum-Annelida)

Hirudin is secreted from leech which retards the coagulation of blood.It is employed as an anticoagulant, in surgical operations and has been recommended for prevention of phlebitis and post operative pulmonary inflammation.

Leech extract is reported to be efficacious in the treatment of asthma, acute rhinopharyngitis and spasmodic coryza.This extract contain mucolytic enzyme.

2:Honey bee (Genus-Apis,Class-Insecta):

Honey is produced by Honey bee. People commonly use honey for burns, wound healing, swelling and sore inside the mouth and cough. Honey is very nutritious and is being used as demulcent and laxative especially for children.

3:Red ants (Chintee,Class-Insecta): Chintee eggs form a constituent of a medicine used for malaria by some tribals.

4: Mulberry silk worms(*Bombyx mori*,Class-Insecta): Silkpod or chrysalis is used as styptic, tonic and astringent. It is believed to check profuse menstruation, leucorrhoea and chronic diarrhoea.

5: Cochineal insects (*Dactylopius coccus*, Class-Insecta): Possesses aphrodisiac, sedative and antiseptic properties and is used in neuralgia and whooping cough.

6:Stink bug (Class-Insecta): The stink bug is used as aphrodisiac in China.

7:Termite queen (Class-Insecta): Termite queen is believed to be powerful sexual tonic.

8: *Mylabris chicorii*(Class-Insecta) *Mylabris chicorii* and other species are used as a substitute for cantharides. It acts as an internal stimulants and diuretic. It is used externally as counter irritant and vesicant.

9-Water beetles(Class-Insecta): Water beetles are reported to be anti-diuretic.

10-Sacred chank (*Turbinella pyrum*,Phylum-Mollusca): It is widely used in the indigenous system of medicine. After burning, the residual lime is used for dyspepsia, piles, general debility and some skin and lung diseases. The calxed shell (bhasma) of chank is used as demulcent and cardiac stimulant. The soft part of the chank are used for the treatment of enlarged spleen.Cowries such as money Cowries (*Cypraea moneta*) are also used as medicine more or less in similar treatment like chank.

11-Apple snail's (*Pila globosa*, Phylum-Mollusca): Its flesh is used for sore eyes in South India.

12-Windowpane Oyster (*Placuna placenta*,Phylum-Molluca): Produces seed pearls which are used in the treatment of eye diseases.

13-Edible Oyster (*Crassostrea madrasensis*,Phylum-Mollusca):Its flesh is used as demulcent.

14-Sea mussels (Phylum-Mollusca): Sea mussels are used in manufacture of vitamin products.

15-Black-lipped pearl Oyster (*Pinctada margaritifera*,Phylum-Mollusca): Produces Moti which is used in the form of Bhasma.

16-Cuttle fish(Phylum-Mollusca): Cuttle fish bone is a hard,brittle internal structure (an internal shell) found in all members of the family-Sepiidae, commonly known as cuttle fish.

Cuttle fish bones of Sepia are used as antacid, astringent and sedative.

17- Shark and Cod fishes(Class-Pisces): Shark and Cod liver oils are used in manufacture of a number of medicines.

18-Cobra Venom (Neurotoxic,Class-Reptilia): Cobra snake venom is an important constituent of a number of medicines in Ayurveda. With fresh juice of sugarcane it is given in the treatment of ascities. It is said to be a bowel irritant, has a purgative action and is used as hepatic stimulant.In small doses Cobra venom is used in neuritis, neuroleprosy, spinal tuberculosis, migraine, intracetable pain due to cancer, arthritis, epilepsy and haemorrhage of retina, uterus and other types of internal bleeding.The dilute solution of Cobra venom (Cobroxin) are reported to cure certain types of cancer.This medicinal property is possessed by a non toxic protein component of venom which destroys certain types of tumour cell in vivo and in vitro.This observation has given up a new directions for research on the surface membranes in biological systems. Another protein isolated from cobra venom helps the nerves fibres in growing faster. Cobra cardiotoxin has been found to have beneficial effect in branchial asthma, pain caused by injury to nerves, neuralgia, rheumatism, hypertonic diseases, arthritis and sciatica.

19-Viper Snake Venom(Haemotoxic,Class-Reptilia): Because of the preponderance of the coagulating principle it is being used as haemostatic agent in haemophilia and in haemorrages due to dental or any other surgery. A nontoxic protein isolated from Russell's viper venom (stypven) has been successfully employed in anticoagulant therapy.

20- Flesh of Peacock (Class-Aves): Flesh of peacock has high medicinal value.Peacock flesh in clarified butter considered efficacious in the restoration of virility and also in curing various diseases.Cock flesh along with flesh of other animals and certain Ayurvedic preparations cures asthma and hiccups.

21-The meat of Sparrow,Cuckoo and Peacock (Class-Aves): It is used as remedy in a number of mental diseases.The cuckoo meat is most useful in treating epilepsy, fainting, hysteria, insanity and senselessness resulting from the excessive drinking. It is also used to restore energy,strength,vitality and increases intelligence and semen production.

22-Bile of Peacock (Class-Aves): The bile of Peacock and its preparations purify blood and also used as an antidote to poison.

23-Pigeon's meat (Class-Aves): The meat of Pigeon is used in treatment of the persons suffering from Paralysis.

24-The Flesh of common Crow pheasant or cuocal (Class-Aves): It is considered as panacea for consumption,asthma and other pulmonary diseases.

25-Flesh and bones of Indian born owl and Andman born owl (Class-Aves): These are highly curative for paralysis, gout and rheumatism.

26-Grey horn bill (Class-Aves): Grey horn bill has high medicinal value. The internal administration of a broth prepared with the entire bird (feather included) and bathing the patients with its decoction is prepared to cure post child birth pains in Women.

27-Bones of horn bill (Class-Aves): Bones of horn bill are also reported to be of great medicinal value.

28-Oil extracted from birds: The oil of pelican fat is highly useful in rheumatism and similar ailments.Peacock fat oil is very much useful in treatment of gout,rheumatism and arthritis.The oils obtained from great Pied Horn bill and Rufousne Horn bill also cure gout and rheumatism.

29-Peacock feathers: Peacock feathers are wrapped around the septic wounds to cure them. The smoke of feathers is considered as antidote to snake bite.Ocellated feathers are used for ophthalmic diseases.

30-Civet(Class-Mammalia): Civet is an odorous secretion obtained from Civet Cat (Viverra, Moschothera and Viverricula) belonging to carnivora.When Civet is applied externally it removes localised pain and itching sensation.It is useful medicine in scabies,pimples and other skin infections.When applied internally it is useful in insanity, mental retardation, fainting and convulsions. Civet is also used in asthma, cough, colic pain and some other respiratory troubles. It is applied in cardiac weakness and removes pulpitation of heart.

31-Pig and cowpancreas (Class-Mammalia): Insulin is manufactured from the pancreas of cow and pig obtained from slaughter house.

32-Liver of Animals: Heparin is used as anticoagulant and prepared from the liver of animals.Now a days it is prepared from the Bovin lungs. It is very frequently used for cases of coronary diseases.In the preparation of B-complex the liver of animal is used.

33-Ox bile (Class-Mammalia): It is used as cholagogue for stimulating flow of bile.

34-Sex glands: The sex glands like testicular extract and ovarian preparations are used for treatment against hormonal deficiency in human body.

35-Aphrodisiacs: These are prepared by a lot of animal products.Even the horn of rare animal Rhinoceros is used for the preparation of aphrodisiacs.

36-Capsules: The capsules are made of gelatin which is obtained by boiling the horns,hoofs,skin,tendons and bones of animals. Now a days capsules are used by all the pharmaceutical industries.

37: Brain of Sheep (Class-Mammalia): For the preparation of anti-rabies Vaccine,the brain of sheep is used.

38-Antivenin: Antivenin and other types of sera are manufactured with the help of Snake venom which is obtained from the tamed snake. The snake venom is further processed on horses in graduated and increasing doses.

39-Musk Deer (Class-Mammalia)-Musk is obtained from musk deer. It is used for the preparation of medicines in Ayurvedic system.

D: Advancements in Pharmaceuticals

The WHO has planned to send a team of experts, at sites where diseases in epidemic form are expected, to study the situation and their control at International level. In 1974 Cholera was in its epidemic form in Portugal from where it spread in Europe through the tourists. The strain of Bacteria was critically examined at the Robert Koch Institute, Berlin and the report was communicated to WHO in Geneva and thus proper steps were taken by the team of experts to control the Cholera epidemic.

During past 20 years of scientific development, biotechnology has revolutionised the era of biological research particularly in the field of medicine, agriculture, pollution control, food processing, biochemical industry, fermentation technology and genetically designed micro-organisms in different field. The industries based on pharmaceutical processings are already producing medicines like hormones and various types of vaccines obtained from genetically designed micro-organisms. About 25% of the total drugs produced in the world are derived with the help of biotechnology.

The use of human hormones as medicines is increasing day by day. The Isolation of human hormone at commercial scale is very difficult process. But the advent of biotechnology has solved the problem a lot as a result sufficient supply of hormones, for therapeutic use, is being managed by pharmaceutical industries. The first recombinant therapeutic human insulin hormone, Humulin was produced at commercial scale is San Francisco in 1983. Further a number of genetically engineered and biotechnologically evolved therapeutic hormones like Protropin, Epidermal growth factor(EFG), Erythropoietin, Fibroblast

growth factor,Nerve growth factor and platelet derived growth factor hormones are produced. Some other therapeutic hormones like Beta and Gamma interferan, relaxin, interleukin, prolactin, inhibin, somatostatin, calcitonin, enkephalins, dynorphin and endorphins are also produced in pharmaceutical biotechnology laboratory which would be great help to human health.

The enzymes are of great significance for the treatment of human beings in a number of diseases. But such enzymes can not be synthesized in general chemical laboratory. The enzymes of medicinal importance are being extracted from human urine, organs, blood or from micro-organisms. They are further produced biotechnologically and given to fulfil the requirement of a particular enzyme deficient in human body.A protein tissue plasminogen activator (t-PA) has been produced biotechnologically which is found in minute quantity in normal human blood system. This enzyme dissolves blood clots in the coronary artery and restore the normal circulation of blood in the heart muscles of human beings. Superoxide dismutase (SOD) is also a biotechnologically produced enzyme which prevents tissue perfusion damage caused due to cut. SOD is also being used possibly with t-PA after the heart attack. Vaccines are gaining more and more importance for preventing a number of diseases. Now a days the genetically engineered and biotechnologically produced vaccines are more preferred than to the conventional vaccines which may occasionally cause the disease they are designed to prevent. The biotechnologically produced vaccines consist only of the antigen portion of the disease causing agent. The genetically engineered and biotechnologically produced vaccines against ‘human hepatitis B' has opened the way to obtain vaccines against a number of fatal diseases.

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The PI3K/Akt Signaling Pathway in Type 2 Diabetes Mellitus

Dr. Deepa Rajendiran[1], Dr. Subbulakshmi Packirisamy[2] and D.E. Nirman Kanna[3]

[1]Associate Professor, Department of Biochemistry, Madha Dental College, and Hospital, Kundrathur, Chennai, Tamil Nadu.

[2]Assistant Professor, Department of Pharmacology, Meenakshi Ammal Dental College, Meenakshi Academy of Higher Education & Research, Chennai, Tamil Nadu.

[3]Perfusionist, First year PG, Department of Cardio Thoracic Surgery, Narayana Hrudayalaya Foundation, Bangalore, Karnataka, India.

Corresponding Author: Dr. Deepa Rajendiran M.Sc., M.Phil., Ph.D. Associate Professor, Department of Biochemistry, Madha Dental College and Hospital Kundrathur, Chennai, Tamil Nadu, India.

E-mail: *deepanish1981@gmail.com*

Abstract

Type 2 diabetes mellitus is a metabolic disorder characterized by insulin resistance, leading to elevated blood glucose levels. The PI3K/Akt signaling pathway is a key molecular pathway implicated in the regulation of glucose uptake and metabolism in various tissues, including muscle, liver, and adipose tissue. In the context of type 2 diabetes, impaired insulin signaling disrupts the normal activation of PI3K/Akt pathway components downstream the insulin receptor. This disruption results in reduced translocation of glucose transporter GLUT4 to the cell membrane, diminishing glucose uptake in insulin-sensitive tissues. Furthermore, dysregulation of the PI3K/Akt pathway contributes to aberrant hepatic glucose production and adipose tissue dysfunction, exacerbating hyperglycemia and insulin resistance. Understanding the intricate interplay between insulin signaling and the PI3K/Akt pathway is essential for developing targeted therapeutic strategies to manage type2 diabetes and its associated complications.

Keywords: Type 2 diabetes mellitus, insulin signaling, PI3K/Akt pathway

Introduction

Type 2 Diabetes Mellitus (T2DM) is a complex, genetic and heterogeneous disease characterized by hyperglycemia [1].According to International Federation of Diabetes (IDF), globally 415 million people in the age group of 20–79 years (8.8% of adult) have diabetes especially 90% of these are type 2 diabetes mellitus and are expected to raise to 640 million by 2040[2]. Based on IDF Report, in 2019, the three countries with the highest number of people with diabetes were China (116.4 million), India (77.0 million) and the United States (31.0 million)[3]. The noncommunicable diseases (NCDs) accounted for 74% of global fatalities in 2019, with diabetes accounting for 1.6 million deaths, making it the ninth biggest cause of death worldwide [4].Over the past three decades, the worldwide burden of diabetes has gradually increased, with India bearing a substantial share of this burden. Disease patterns in India show change due to epidemiological transition:As a result, mortality from infectious, maternal, neonatal and nutritional diseases has decreased significantly, while non-communicable diseases and injuries have significantly increased their contribution to disease burden and overall mortality [5].

Type 2 Diabetes Mellitus is one of the major chronic disorders in the world, and its development is mainly due to a combination of two major factors: insulin-secreting pancreatic β-cells are defective and insulin-sensitive tissues are incapable of responding to insulin [6]. The release and action of insulin must precisely respond to metabolic needs; therefore, the molecular mechanisms involved in insulin synthesis and release, as well as the insulin response in tissues, must be tightly regulated. Therefore, any defects in the regulation can leads to a metabolic disturbance leading to the pathogenesis of T2DM [7].

Lipid kinases is an enzyme which phosphorylates the inositol portion of phospholipids at the 3rd position, are assigned to the phosphatidylinositide 3-kinase (PI3K) superfamily. They play important role in the physiological control of body homeostasis but also targeted for drug therapy for a wide range of human diseases (8-10). These proteins have been shown to be important molecules for glucose homeostasis and, therefore, their dysregulation may be associated with increased serum glucose concentrations, which is characteristic of the most important pathophysiology of diabetes mellitus. PI3K is strongly associated with diabetes-induced target organ damage, including blood vessels, heart, and brain [11]. This review article mainly focuses the insulin signaling pathway in type 2 diabetes.

The insulin signaling cascade

Insulin is a hypoglycemic hormone secreted by pancreatic beta cells in response to rises in blood glucose levels. After being secreted, insulin travels through the bloodstream and circulates until it attaches to the insulin receptor on a cell surface [12]. When insulin attaches to its receptors on target cells, such as skeletal muscle cells and adipocytes, a signaling cascade is initiated, followed by receptor autophosphorylation, and activation of receptor tyrosine kinases, resulting in tyrosine phosphorylation of insulin receptor substrates (IRSs) including IRS-1, IRS2, IRS-3, IRS-4 [13]. Binding of insulin receptor substrates to phosphoinositide 3-kinase (PI3K) leads to PI3K activation.Activated PI3k kinases are involved in the phosphorylation of membrane phospholipids and phosphatidylinositol 4,5-bisphosphate (PIP2). which in turn activates 3-phosphoinositide-dependent kinases (PDK-1 and PDK-2), leading to Akt/protein kinase B (PKB) activation (14.15). The PI3K/Akt pathways is an intracellular signaling pathway which plays a central role in cell physiology by regulating growth factor signals during organismal development and important cellular processes, such as glucose homeostasis, protein synthesis, lipid metabolism, proliferation and cell survival in response to extracellular signals [Fig.1][16].

PKB/Akt is a serine/threonine kinase containing a pleckstrin homology domain, a catalytic domain, and a putative moiety at the carboxyl end. PKB/Akt activation includes contact with PIP3 and/or PIP2 via phospholipid binding at the pleckstrin homology domain. Akts are classified into three isoforms based on variations in serine/threonine residues (Akt1, Akt2, and Akt3). Akt1 is found ubiquitously, Akt2 is found mostly in insulin-sensitive tissues such skeletal muscle, adipose tissue, and liver, while AKT3 is found in the testes and brain[17,18]. Three PKB/Akt isoforms encoded by three distinct genes have been identified in mammalian cells.Over the past decade, research has found specific roles for each isoform, in which Akt1 is associated with cell survival, Akt2 has been linked to cell-substrate metabolism[19], and Akt3 has been linked to brain development[20]. Akt is mainly activated by insulin in muscle tissue, liver, and adipocytes[21].

Akt signaling is important in insulin-stimulated glucose uptake in both muscle and adipose tissue, which inhibits the glucose release in liver (gluconeogenesis and glycogenolysis) [22]. It is achieved via Akt by translocating GLUTs(Specific glucose transporter) to the cell membrane, hence facilitating glucose uptake. As insulin binds to its cell surface protein receptors, phosphorylation of insulin receptor substrates in specific tyrosine residues

occurs, as does activation and recruitment of PI3 kinase and its downstream target Akt/PKB[23].

The main targets of activated PKB are GSK-3 and AS160. AS160, the Akt substrate is also known as TBC1D4 which regulate GLUT-4 translocation in skeletal muscle [24]. AS160 maintains the Rab-GTPase(s) in sedentary form by loading them with guanosine-50-diphosphate, which keeps GLUT-4 in the GLUT storage vesicles [25,26]. Once activated, Akt/PKB phosphorylates reduce AS160, leading to a decrease in Rab-GAP activity, promoting GLUT-4 translocation and glucose uptake.Therefore, any defects in the PI3 kinase/Akt/AS160 transduction pathway will eventually reduce glucose uptake in skeletal muscle. Similarly, the deletion of Akt1 and Akt2 isoform in liver causes glucose intolerance and insulin resistance [27].

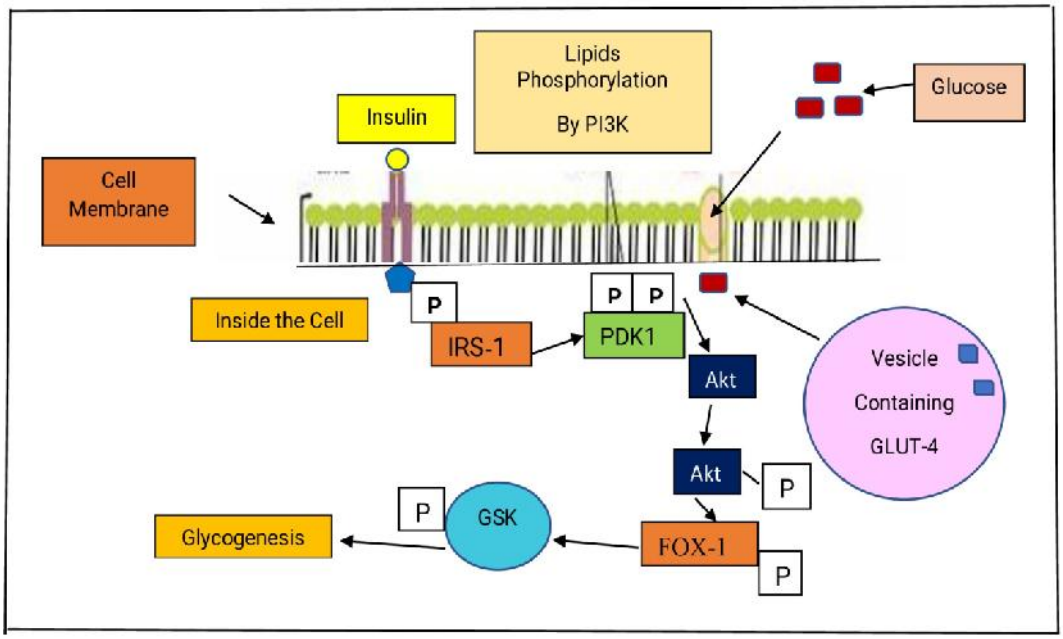

Figure 1: Insulin Signaling pathway

Akt/PKB and insulin resistance

Insulin resistance is physiologically defined as a condition of reduced response in insulin-targeted tissue to higher insulin levels, and is considered to be the pathogenic driver of many diseases such as T2DM, atherosclerosis, and non-alcoholic fatty liver diseases (NAFLD)[28]. The main clinical sign of T2DM is non-physiologic high blood glucose levels, which are preceded by insulin resistance.In prediabetes, insulin levels rise to satisfy normal insulin

demands, resulting in chronic hyperinsulinemia, hyperglycemia-induced-cell failure, and, finally, T2DM[29]. As mentioned above,the cellular uptake of glucose, glycogen synthesis and decrease of hepatic glucose production are not observed in insulin resistant tissues.

PI3K/AKT pathway in Liver

Hepatic insulin resistance may be caused by defective signaling via the insulin receptor substrate (IRS) proteins which connect insulin receptor activation to important downstream kinase cascades such as the PI3K or MAPK pathways. IRS-1 and IRS-2 are extensively expressed in normal mice livers but are downregulated to varying degrees in diabetic animal liver [30,31]. Insulin signaling is a critical mechanism in the liver for regulating glycogen metabolism, gluconeogenesis, and lipogenesis [32]. These processes are controlled by the phosphorylation and dephosphorylation of many proteins involved in metabolic pathways, the expression of numerous genes, and the stability of mRNA[33].

Studies of insulin receptor substrates and signaling in the *ob/ob* mouse, an insulin resistance model of obesity and non-insulin-dependent diabetes, have reported that lower expression levels of both receptor substrates insulin-1 and 2 are reduced by about 50% in muscle, whereas in liver the decrease was significantly greater for insulin receptor substrate-2 (72%) than insulin receptor substrate-1 (29%) [34]. IR protein and mRNA levels in rat liver were significantly reduced in a type 2 diabetes model, which has been demonstrated by experimental studies [35]. Moreover, IRS-1, PI3K, and Akt exhibited the similar trend as IR. Decreased Akt levels maydecline the release and transport of glucose transporter into vesicles. In another study, glycogen content in the liver of type 2 diabetic rat decreased due to interference of GSK3-mediated liver glycogen synthesis.It is due to reduced insulin-PI3K signaling in type 2 diabetes mellitus[36].

PI3K/AKT pathway in Muscle

Skeletal muscle in persons with T2DM shows a variety of pathological alterations that affect glucose transport, absorption, and metabolism.Glucose transport is a rate-limiting step for glucose utilization under physiological conditions in skeletal muscle [37]. The decreased disposal of non-oxidative glucose was observed in people with metabolic syndrome, obesity, and type 2 diabetes [38].Alteration in skeletal insulin signaling in type 2 diabetes mellitus condition leading to impairment of GLUT-4 protein translocation to the membrane [39] [Fig.2].The insulin-stimulated phosphorylation of AS160 is a

critical step in GLUT4 translocation and has been shown to decrease inpatients with type 2 diabetes [40]. Zaid et al. [41] reported that GLUT4 mRNA expression is reduced in type-2 diabetic subjects, due to defective transcription of the GLUT4 and changes in the stability of its mRNA transcript.

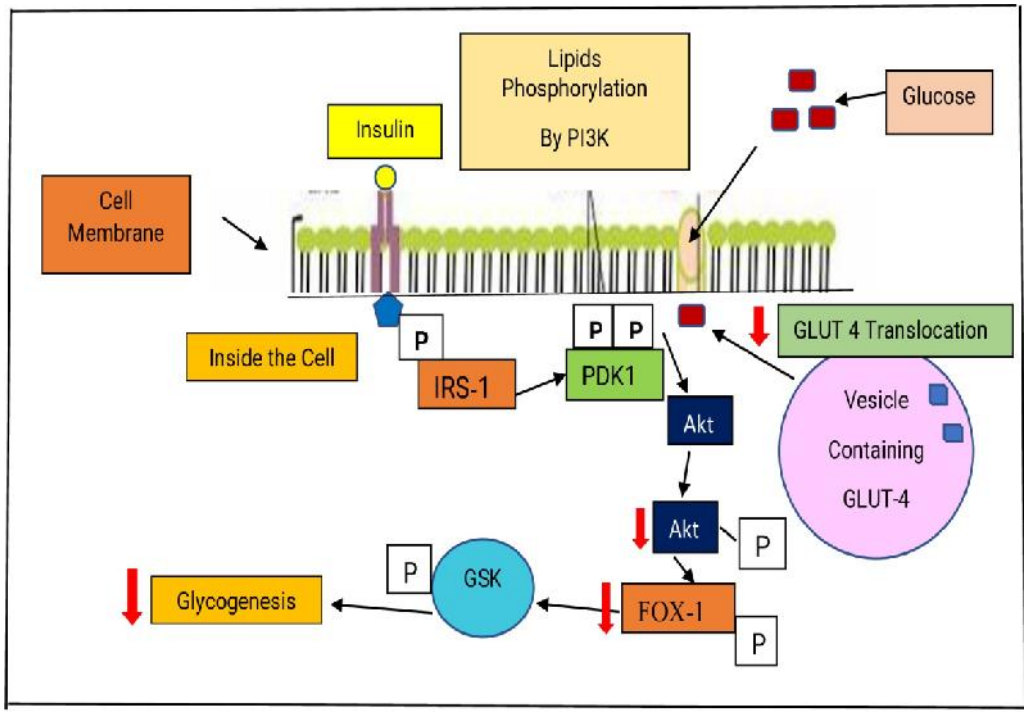

Figure 2: Insulin Signalingpathway in Type 2 Diabetes mellitus

Conclusion

Insulin signaling play an important role in the regulation of metabolism such as carbohydrates, proteins and lipids.PI3K/Akt is the primary signaling pathway which is found in many organs and plays a key role in a variety of physiological activities.Insulin resistance in type 2 diabetes is caused by abnormalities at one or more levels of the insulin-signaling cascade in skeletal muscles, adipose tissue, and the liver. Therefore, the signaling pathway in the liver, muscle and beta cell is emerging as a key factor in preventing type 2 diabetes.The understanding of this pathway is critical for the development of novel medications to treat diabetes and associated disorders.

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Exploring the era of essential oils against Oral pathogens – Revisited

Dr. A. Kiruthiga
Associate Professor in Microbiology,
Madha Dental College and Hospital
(Affiliated to The T.N. Dr. M.G.R. Medical University),
Kundrathur, Chennai – 600 069
Phone Number: 7010921118
Email: *kiruthigaalexander@gmail.com*

Abstract

The oral microbiome, constituted by bacteria, fungi, viruses and parasites, serves as a potential marker denoting the overall health status of an individual. Antibiotic therapy helps in the active management of bacterial infections in the oral cavity. Inappropriate use of antibiotics led to the emergence of infections caused by antibiotic-resistant bacteria. Addressing the availability of fewer antibiotics in the pipeline, essential oils have gained significance as an alternative treatment for substituting traditional antibiotic therapy. Also, recent studies have established the antimycotic, antiviral and antiparasitic activity of essential oils. This paper reviews the antimicrobial efficacy of essential oils targeted against oral pathogens.

Keywords: Oral microbiome, Essential oils, antimicrobial efficacy, antibiotic resistance

Introduction

Historical perspective regarding microbial existence as an integral part of humans dates back to the second half of the 16th Century by prodigy Antonie Van Leeuwenhoek following an examination of his dental plaque sample [1]. The human oral cavity serves as a potent interacting junction that exists between the body and the external environment [2].

Various niches such as teeth, tongue, tonsils, hard and soft palate and gingival crevices available within the human oral cavity form the favorable colonization spot predominantly for the bacteria constituting the oral microbiome [3]. Microbes present in the oral cavity of humans constitute the oral microbiota [4].

Oral microbiota is considered the second largest microbial community next to gut microbiota among humans [5]. It constitutes a plethora of microorganisms of more than 1000 bacterial species and over 100 species of fungi [6], viruses and protozoans [7].

Human oral microbiome:

A healthy human oral microbiome majorly constitutes the Gram-positive bacterial colonizers such as *Abiotrophia, Peptostreptococcus, Streptococcus, Stomatococcus, Actinomyces, Bifidobacterium, Corynebacterium, Eubacterium, Lactobacillus, Propionibacterium, Pseudoramibacter, Rothia* and Gram-negative bacterial colonizers *Moraxella, Neisseria, Veillonella, Campylobacter, Capnocytophaga, Desulfobacter, Desulfovibrio, Eikenella, Fusobacterium, Hemophilus, Leptotrichia, Prevotella, Selemonas, Simonsiella, Treponema, Wolinella* [5].

Oral bacteria can serve as opportunistic pathogens with the modifications of the host and microbial interaction resulting in infection [8]. Bacterial members either exist in the planktonic form or may adhere to the surfaces of the oral cavity and form a plaque or biofilm, majorly responsible for the pathology including systemic diseases [9].

Infections in the oral cavity and their significance:

Infections caused by oral bacteria may not be confined to the oral cavity but can also be responsible for Alzheimer's disease, osteomyelitis, atherosclerosis and even diabetes and endocarditis. These infections were effectively managed with antibiotics and accompanied by the rapid emergence of antibiotic-resistant strains.

Estimation of CDC's 2019 Antibiotic resistance threat report accounts for the national death and infection caused by 18 antimicrobial-resistant bacteria and fungi. This report highlighted the rapid evolution of multidrug-resistant bacterial strains posing a potential global threat with fatal cases of about 1.27 million people worldwide. This complicates the available therapeutic strategies and the search for an alternative ideal antibacterial agent was intensified [10].

Commonly encountered oral pathogens:

The most commonly encountered oral pathogens include Gram-positive cocci such as *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus sanguis*, *Streptococcus sobrinus*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Enterococcus faecalis* and yeast-like fungi *Candida albicans* and

Actinobacillus actinomycetemcomitans responsible for oral dysbiosis and development of resistance for the other identified therapeutic strategies [11,12]. Commonly occurring oral diseases include caries, periodontal disease, pulp periapical disease, oral cancer, recurrent oral ulcer and peri-implantitis [6].

Evolution of essential oils – a potential alternative therapy against the oral pathogens:

Natural aromatic essential oils derived from different parts of plant extracts have been traditionally used in folk medicine in many Countries for over 100 years [13,14]. Essential oils have been extensively used by the Egyptians since 3500 BC for medicine, cosmetics and religious events [14]. The term 'Essential oil' was named by Paracelsus von Hohenheim in the 16th Century referring to the effective component of the drug "Quinta essentia" [15]. Essential oils have been considered significant since their first usage in the East and Middle East, followed by their wide utilization in Europe and North Africa [13].

Of the 3000 essential oils known so far, 300 are commercially available and widely employed in various industrial fields such as cosmetics, food preservatives and pharmaceutical products including dentistry and medicine [16]. Conferring to the World Health Organisation, 80% of the population from developing nations use herbal medicines to meet their fundamental medical attention [17]. Recent research works have targeted utilizing essential oils as a novel alternative therapy against the pathogens present in the oral microbiome [18].

Consequently, during the search for natural agents combating antibiotic resistance, Essential oils (EO) have gained potency as a novel therapeutic agent because of their antimicrobial activity against bacteria, fungi and viruses. They are evidenced as a promising alternative strategy in the treatment of serious infections caused by antibiotic-resistant strains of bacteria [19].

Commonly used essential oils against oral pathogens:

Commonly employed essential oils against oral pathogens include lavender oil, eucalyptus oil, peppermint oil, tea tree oil, clove oil, lemon oil, cinnamon oil and coconut oil [20, 21].

These oils possess bactericidal action against antibiotic-resistant bacteria including *Streptococcus mutans*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus sanguis*, *Streptococcus anginosus*, *Actinobacillus actinomycetemcomitans*, *Streptococcus sobrinus*,

Staphylococcus epidermidis, Escherichia coli, Prevotella intermedia, Porphyromonas ginigivalis, Fusobacterium nucleatum [22] and oral spirochaete *Treponema denticola* [23], antiviral activity against herpes simplex viruses type 1 and arboviruses [24, 25], antiparasitic against *Trypanosoma cruzi*, *Leishmania brasiliensis*, *Plasmodium falciparum* [25] and antimycotic activity against yeast-like fungi *Candida* species, molds [26] and other dermatophytes [27].

Sources of essential oils:

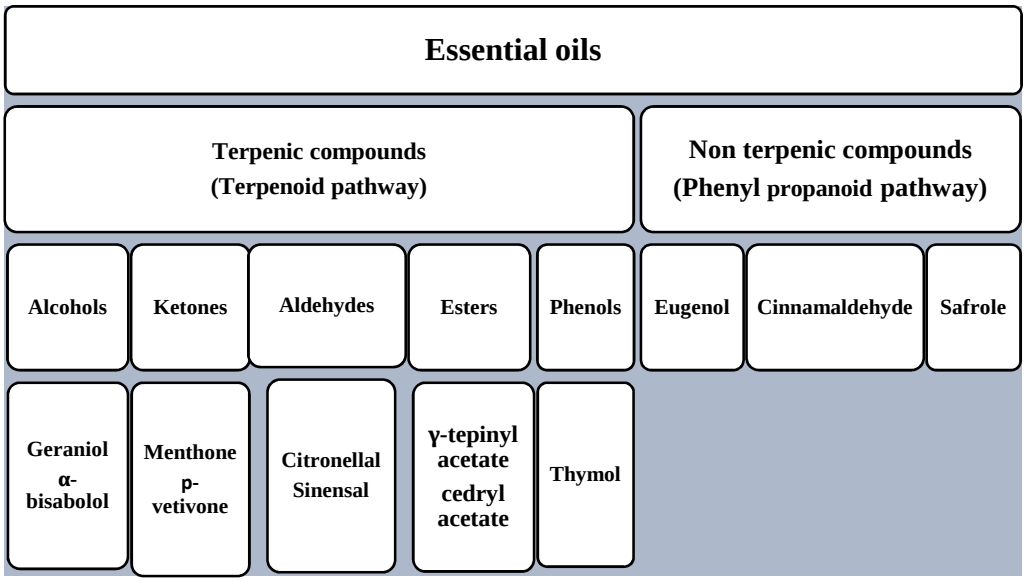
Aromatic plants confined to temperate and tropical areas of Countries, representing a significant area of classical pharmacopeia, are the target sources of essential oils. They are the volatile substances synthesized from different organs of the plants including roots, stem, leaves, flowers, fruits, buds and also from various storage areas like canals, secretory and epidermal cells, cavities and glandular trichomes or from the whole plant [16, 28].

They are usually liquid in consistency and remain colourless at room temperature. They are presented with a characteristic odour and are soluble in lipids and other organic solvents, having decreased density when compared with water [29].

Composition of essential oils:

Essential oils are intricate blends composed of over 300 chemical composites predominantly terpenes, terpenoids, aromatic hydrocarbons, alcohols, acids, esters, aldehydes and ketones such that their antibacterial activity cannot be confined to a single compound [30] (Fig.1). These oils are complex mixtures that may contain over 300 different compounds [31].

Figure 1: Principal chemical constituents of essential oils



Mechanism of action:

Characteristic antimicrobial properties of essential oils may be attributed to two stages i.e., either microbiostatic or microbicidal. However, the effectiveness of essential oils can be majorly influenced by their chemical structure, the conditions of the prevailing environment as well the targeted bacterial cell wall [26]. The mode of action of the antibacterial activity of essential oils can be mediated by various factors such as altering the permeability of the cell wall thereby inducing the leakage of intercellular material, destruction of the outer and inner membrane, loss of membrane integrity, cytoplasmic changes finally resulting in the disruption of the cell membrane and cell wall while the antifungal activity can be mediated by the inhibition of biosynthesis of ergosterol in the fungal cell wall thereby disrupts the membrane integrity [32]. The active components, mode of action and the binomial nomenclature of the commonly used essential oils against oral pathogens are summarised in Table 1

Table 1. Active components of commonly used essential oils against oral pathogens

Essential oil	Binomial nomenclature	Active components	Mode of action	Reference
Lavender oil	*Lavandula augustifolia Lavandula stoechas* L.	Linalool, Linalyl acetate, lavandulol, 1,8-cineole, lavandulyl acetate and camphor.	Antibacterial, Antifungal, Antiviral	[33]
Eucalyptus oil	*Eucalyptus globulus*	1,8-cineole, cryptone, α-pinene, *p*-cymene, α-terpineol, trans-pinocarveol, phellandral, cuminal, globulol, limonene, romadendrene, spathulenol and terpinene-4-ol	Antibacterial	[34]
Peppermint oil	*Mentha piperita*	Menthol, Menthyl acetate and menthofuran	Antifungal	[35]
Tea tree oil	*Melaleuca alternifolia*	Terpinen-4-ol, γ-terpinene, *p*-cymene, α-terpinene, 1,8-cineole, α-terpineol and α-pinene	Antiparasitic	[36]
Clove oil	*Syzygium aromaticum L*	Phenylpropanoids eugenol, eugenyl acetate, carvacrol, thymol, cinnamaldehyde, β-caryophyllene and 2-heptanone	Antibacterial, antifungal, antiviral	[37]
Lemon oil	*Citrus limonum*	Terpenes and oxygenated terpenes	Antifungal	[38]

Cinnamon oil	*Cinnamomum zeylanicum*	*Trans*-cinnamaldehyde, eugenol and linalool	Antibacterial	[39]
Coconut oil	*Cocos nucifera*	Oleic acid, fatty acids, capric acid, palmitic acid, caprylic acid, lauric acid, stearic acid, myristic acid and linoleic acid	Antibacterial, Antifungal	[21]

Antimicrobial efficacy of essential oils:

The antimicrobial efficacy of essential oils depends on their composition as well as the magnitude of the interaction between the volatile constituents present in them [40]. Essential oils possess significant antibacterial activity against Gram-positive bacteria than Gram-negative bacteria [41, 42]. The lipopolysaccharide layer and the Outer membrane of the Gram-negative bacteria act as a barrier for the hydrophobic compounds limiting their diffusion, is the prime reason for the resistance of Gram-negative bacteria towards the activity of essential oils [43].

Therapeutic applications of essential oils:

Therapeutic applications of essential oils are considered significant as they possess i) Highly diffusible and penetrating potential, ii) Disinfecting and strengthening the immune system, iii) Stimulation and maintenance of the functional equilibrium, iv) Regulation of the neuroendocrine functions, v) Psychosomatic effect and influence on the Central nervous system [44].

Relevant to the field of dentistry, essential oils find several therapeutic applications in relieving pain, in the effective treatment of oral candidiasis and against cariogenic bacterial pathogens and prevention of gum diseases [45]. Also, they are widely employed as preservatives, mouthwashes, tranquilizers, dental implants and adjuncts maintaining oral hygiene [46].

Limitations:

Major limitation associated with the utilization of essential oils can cause an imbalance with the commensals constituting the oral microbiome thereby leading to the emergence of new infections or diseases in the oral cavity [47]. Though the antimicrobial properties of essential oils against oral pathogens are acknowledged, it is essential to accomplish the equilibrium of

microbes using various combinations of essential oils [46]. Researches monitoring the safe precautionary measures of intraoral intake of essential oils still needs to be explored for their broad spectrum of application in dentistry (Hans et al, 2016) [48].

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Physico-chemical characteristics for testing water

Prof. Indu Singh[1] and Dr.R.B.Tripathi[2]
[1]P.G. Department of Zoology, K.N.I.P.S.S. Sultanpur-228118, (U.P.), India
[2]P.G. Department of Zoology, M.L.K.P.G.College Balrampur-271201(U.P.), India
Email: *desiredindu@gmail.com* & *drrbtripathi.77@gmail.com*

Introduction

Water is one of the most important and essential compounds of the ecosystem. All living organisms on the earth need water for their survival and growth. As earth covers 70% of water, hence it is some time well known as water planet. But due to increased human population, industrialization, use of fertilizers and man-made activities, it gets highly polluted. Therefore it is necessary that the quality of drinking water should be checked at regular time interval, because such polluted water is unfit for human population, as a result human suffers from different water borne diseases such as typhoid, cholera, dysentery, hepatitis etc. It is difficult to understand the biological phenomenon fully because the chemistry of water reveals much about the metabolism of the ecosystem and explain the general hydro-biological relationship.

The availability of good quality water is an indispensable feature for preventing diseases and improving quality of life. Natural water contains different types of impurities are introduced into aquatic system by different ways such as weathering of rocks and leaching of soils, dissolution of aerosol particles and from several human activities. People on global scale are under tremendous threat due to undesired changes in the physical, chemical and biological characteristics of air, water and soil. These are related to fauna and flora and finally affecting on it. Many industrial development results in the generation of industrial effluents, and it results in water pollution as well as soil pollution. High levels of pollutants mainly organic matter in water bodies create and increase in biological oxygen demand, chemical oxygen demand, total dissolved solids, total suspended solids etc. They make water unfit for drinking, irrigation or any other purpose.In many parts of the country available water is rendered non potable because of the presence of heavy metals in excess. This situation gets worsened during the summer season due to water scarcity and rain water discharge. Contamination of water resources available

for household and drinking purposes with heavy elements, metal ions and harmful micro-organisms is one of the serious major health problems.

Most of the rivers in the urban areas of the developing countries are the ends of effluents discharged from the industries. Developed countries experiencing rapid industrial growth and this is making environmental conservation a difficult task.

Physico-chemical Characteristics

These physico-chemical characteristics are very essential and important to test water before used for various purposes. Selection of parameters for testing is depends upon for what purpose water using and what extent we need of water quality and purity. Water contains many contents that include floating, dissolved, suspended, microbiological and bacteriological impurities. Some physical parameters are tested for physical appearance of water such as temp, color, odour, PH turbidity, TDS etc. while chemical parameters are tested for its chemical appearance like BOD, dissolved oxygen, alkalinity, hardness and other characteristics for obtaining more and more quality and purity, water should be tested for its trace metal, heavy metal content and organic residues. It is obvious that drinking water should pass these all tests and it should contain required amount of mineral level. Following some physico-chemical parameters are tested regularly for monitoring water quality.

I: Temperature

The water temperature controls the rate of all chemical reactions and also affects fish growth, reproduction and immunity of fishes.

II: pH

PH is the most important physical characteristics of water. pH is positively correlated with electrical conductance and total alkalinity (Gupta 2009). The reduced rate of photosynthesis, assimilation of carbon dioxide and carbonates are responsible for increase in pH of water. Many factors brings about changes in pH of water, it may be high or low. The higher pH value suggests that CO_2, carbonate, bicarbonate equilibrium is affected more due to change in physico- chemical characteristics.

III: Carbon Dioxide

O_2 is the end product of organic carbon degradation in almost all aquatic environments and its variation is often a measure of net ecosystem metabolism (Smith 1997, 1993). CO_2 is also the most important greenhouse gas on Earth.

There are various measurable parameters of aquatic CO_2 system such as pH (PCo_2), total dissolvedinorganic carbon (DIC) and total alkalinity (TA). Surface water (PCo_2) can be measured by photometric method and DIC CO_2 is measured by coulometer, TA CO_2 is measured by HCl titration of the water sample to the CO_2 equivalence point .

IV: Dissolve Oxygen

DO is also one of the most important parameter. Its correlation with water body gives direct and indirect information of bacterial activity, photosynthesis, availability of nutrients, stratification etc. In summer DO decreased due to increase in temperature and microbial activity. During summer the long days and intense sunlight seems to accelerate photosynthesis by phytoplankton, utilizing CO_2 and giving off O_2. This accounts for the greater qualities of O_2 recorded during summer.

DO is measured titrimetrically by Winkler's method after five days incubation at 293 k. The difference in initial and final DO gives the amount of O_2 consumed by the bacteria during this period.

V: Alkalinity

It is composed of carbonate (Co_3) and bicarbonate (HCO_3). Alkalinity acts as a stabilizer for PH. Alkalinity, PH and hardners affect the toxicity of many substances in the water. It is determined by dilute HCL titration in the presence of phenolphthalein and methyl organe indicators.

VI: Carbonate and Bicarbonate

When the pH of water becomes 8.3, this indicates the presence of carbonates. It is measured by titration method with standard HCl using phenol as indicator. Below the pH of water than 8.3, the carbonates are converted into bicarbonates.

Bicarbonate also measured by titration with standard HCl using methyl organe as indicator. Methyl organe turns yellow below pH 4.0 at this pH, the carbonic and decomposes to give Co_2 and H_2O

VII: Biological Oxygen Demand (BOD)

BOD is a measure of organic material contamination in water, specified in mg/l. BOD is the amount of dissolved O_2 required for the biochemical decomposition of organic compounds and the oxidation of certain inorganic material. The test for BOD is conducted over a five day period (Milacron Marketing Co.)

VIII: Chemical Oxygen Demand (COD)

COD is also a measure of organic material contamination in water in mg/l. It is the amount of dissolved O_2 required to cause chemical oxidation of the organic material in water. Both BOD and COD are key indicators of the environmental health of water. These are commonly used in waste water treatment but rarely in general H_2O treatment. (Milacron Marketing Co.)

IX: Sulphate

It is measured by nephelometric method in which the concentration of turbidity is measured against the known conc. of sulphate solution. Barium chloride is used for producing turbidity due to barium sulphate and a mixture of organic substance and sodium chloride is used to prevent the setting of turbidity.

X:Calcium

It is measured by complex metric titration with standard solution of EDTA using patton's and Reeder's indicator under the pH conditions of more than 12.0. These conditions are achieved by adding a fixed volume of 4N sodium hydroxide. The volume of tire (EDTA soln.) against the known volume of sample gives the concentration of calcium in the sample.

XI: Magnesium

It is also measured by complexometric titration with standard solution of EDTA using Eriochrome black T as indicator under the buffer conditions of PH 10.0. The buffer solution is made from Ammonium chloride and Ammonium hydroxide. The solution resists the PH variations during titration.

XII: Sodium

It is measured with the help of flame photometer. The instrument is standardized with the known concentration of sodium ion (1 to 100 mg/litre). The samples having higher concentration are suitably diluted with distilled water and the dilution factor is applied to the observed values.

XIII: Potassium

It is also measured with the help of flame photometer the instrument is standarilized with known concentration of potassium sol^n, in the range of 1 mg to 5 mg. /litre. The sample having higher concentration is suitably diluted with distilled water and the dilution factor is applied to the observed values.

XIV: Chloride

It is measured by titrating a known volume of sample with standard silver nitrate sol^{n}. using potassium chromate sol^{n}. in water as an indicator. Eosin or fluorescein sol^{n}. in alcohol also used as another indicator. The eosin indicator is an adsorption indicator while the potassium chromate makes a red coloured compound with silver as soon as the chlorides are precipitated from sol^{n}.

Table-1: Different parameters with their analytical technique and guideline values as per WHO and Indian standard

Sr. No	Parameter	Technique used	WHO standard	Indian Standard	EPA guidelines
1	Color	Visual color kit	-	5 Hazen unit	-
2	Odour	Physiological sense	Acceptable	Acceptable	-
3	Temperature	Thermometer	-	-	-
4	PH	PH meter	65.-9.5	65.-9.5	65.-9.5
5	Dissolved O_2	Redox titration	-	-	-
6	Alkalinity	Acid-base titration	-	200 ppm	-
7	Carbonate & Bicarbonate	Titration	-	-	-
8	BOD	Incubation followed by titration	6	30	5
9	COD	COD giester	10	-	40
10	Chloride	Argentometric titration	250 ppm	250 ppm	250
11	Magnecium	Complexo metric titration	150 ppm	30 ppm	-
12	Potassium	Flame	-	-	-

		photometer			
13	Sodium	Flame photometer	200 ppm	180 ppm	200
14	Sulphate	Nephelometer Turbidimeter	250 ppm	200 ppm	250
15	Calcium	Complexometric titration	-	-	-

Ref: WHO, USEPA, Indian Standard, National Primary Drinking Water Regulations, Drinking Water Contaminants USEPA)

Table-2: Different water quality parameters used for testing of quality of water and their source of an occurrence and potential health effects with USEPA guidelines

Sr.No.	Parameters	Source of occurrence	Potential health effect
1	Turbidity	Soil runoff	Higher level of turbidity are associated with disease causing bacteria
2	Color	Due to present of dissolved salts	-
3	Odor	Due to biological degradation	Bad odor unpleasant
4	PH	PH is changed due to different dissolved gases and solids	Affects mucous membrane, bitter taste
5	Dissolved Oxygen (DO)	Presence due to dissolved oxygen	DO corrode waterlines, boilers and heat exchangers at low level marine animals ant surive

6	Total Alkalinity	Due to dissolved gases (Co_2)	Embrittlement of boiler steel, boiled rice turns yellowish
7	TDS	Presence all dissolved salts	Undesirable taste, gastro-intestinal irrigation, corrosion
8	BOD	Contamination due to organic matter	High BOD decreases level of dissolved O_2
9	Calcium	Precipitate soaps, anionic	Interference in dyeing textile
10	Magnesium	Surfactants, anionic, emulsifiers	Paper industry
11	Carbonate	Due to dissolution of Co_2	Productimbalance, unsatisfactory production short product life
12	Chloride	Water additive used to control microbes, disinfect	Eye/nose irritation, stomach discomfort
13	Sodium	Natural component of water	-
14	Sulphate	Due to dissolved ca/mg/Fe sulphates	Taste affected, gastro-intestinal irrigation

Conclusion

A study of physico-chemical parameters for testing water showed that, these parameters are essential for to test water whether water is clear or away from pollution. On these parameters one can come to conclusion, that water is potable for domestic uses as well as for crop fields. Hence the study has taken in to consideration.

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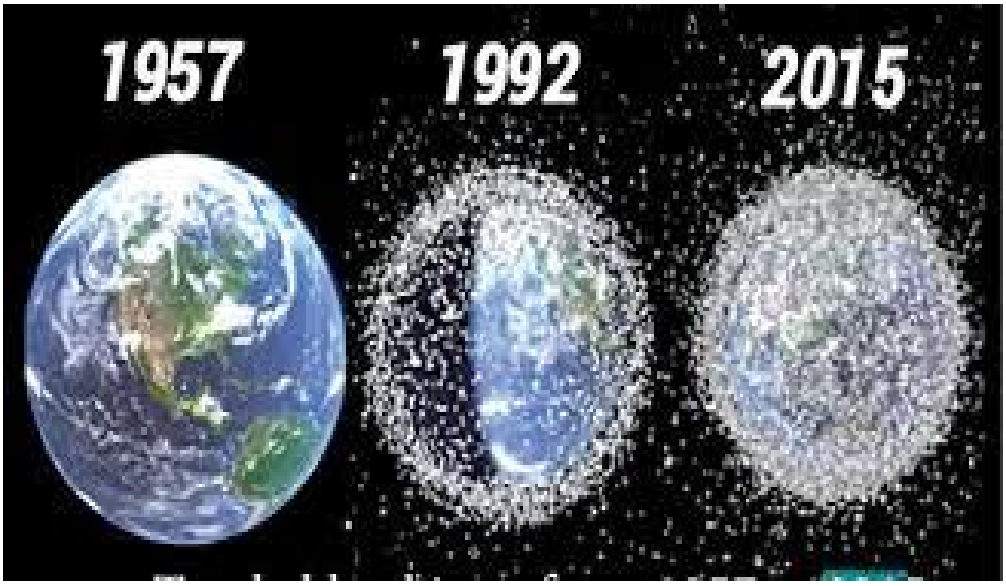
Navigating the cosmos: Unraveling the growing issue of space pollution

Dr. Ashok Kumar
Department of Zoology, M.L.K PG College, Balrampur, (U.P), India

Introduction

In the immense realm of outer space, where the universe extends endlessly in every direction, it might seem natural to envision an untouched and pristine environment. However, the reality diverges significantly from this idealized concept. Even in the far reaches of space, pollution has become an increasingly prevalent issue as humanity's activities expand beyond the confines of Earth. This chapter delves into the various types of pollution in space, the factors causing them, and the potential implications they hold for future space exploration and our home planet.

Space debris, which is also referred to as space junk, space pollution, space waste, space trash, space garbage, or cosmic debris, pertains to non-functional human-made objects in space, primarily located within Earth's orbit, that no longer serve any practical purpose. These objects encompass abandoned spacecraft, non-operational launch vehicle stages, remnants from missions, and especially abundant in Earth's orbit, fragments resulting from the disintegration of abandoned rocket bodies and spacecraft. Apart from these derelict human-made objects left adrift in orbit, space debris includes pieces originating from their breakup, wear and tear, and collisions, as well as minute particles like paint specks, solidified liquids expelled from spacecraft, and unburned remnants from solid rocket motors. Space debris poses a significant hazard to spacecraft



Space debris is essentially an unintended consequence that imposes additional costs on others due to the actions of launching or using spacecraft in near-Earth orbit. These costs are often not considered or fully factored into the expenses borne by the entity responsible for launching or owning the payload.

Numerous spacecraft, both with and without crews, have suffered harm or destruction as a result of encounters with space debris. Some participants in the space industry engage in activities aimed at measuring, mitigating, and potentially removing this debris.

As of November 2022, the US Space Surveillance Network reported the existence of 25,857 artificial objects orbiting Earth, including 5,465 functional satellites. It's important to note that these figures only encompass objects that are large enough to be tracked and located in orbits conducive to tracking. Debris from satellites in Molniya orbits, like the KosmosOko series, may be positioned too high above the northern hemisphere for effective tracking. Furthermore, as of January 2019, it was estimated that there were over 128 million pieces of debris smaller than 1 cm (0.4 in), approximately 900,000 pieces measuring between 1 and 10 cm, and around 34,000 pieces larger than 10 cm (3.9 in) in Earth's orbit. When the tiniest objects of artificial space debris, such as paint flecks and solid rocket exhaust particles, are combined with micrometeoroids, they are sometimes collectively referred to as MMOD (Micrometeoroid and Orbital Debris). Collisions with such debris pose a threat to spacecraft, with even the smallest objects causing damage akin to sandblasting. This is particularly problematic for components like solar panels

and sensitive optics such as telescopes or star trackers, which are difficult to shield effectively against such impacts.

At altitudes below 2,000 kilometers (about 1,200 miles) above Earth's surface, the concentration of space debris is higher than that of meteoroids. This debris primarily consists of fine particles originating from solid rocket motors, surface erosion debris such as paint fragments, and frozen coolant from Soviet nuclear-powered satellites.To put this into perspective, the International Space Station (ISS) orbits at an altitude ranging from 300 to 400 kilometers (approximately 190 to 250 miles). Notably, the two most recent significant events involving space debris—the 2007 Chinese antisatellite weapon test and the 2009 satellite collision—occurred at altitudes of 800 to 900 kilometers (approximately 500 to 560 miles). The ISS is equipped with Whipple shielding to protect it from damage caused by small micrometeoroids and orbital debris (MMOD). However, any known debris with a collision probability exceeding 1 in 10,000 is actively avoided by maneuvering the station to minimize the risk of impact.The issue of space debris accumulation in Earth's orbit began immediately following the launch of the first artificial satellite, Sputnik 1, in October 1957. However, even before this milestone, human activities might have generated ejecta that eventually became part of the space debris population, as illustrated by the Pascal B test conducted in August 1957. Going further back in time, there were instances of natural ejecta from Earth entering orbit as well.

Following the launch of Sputnik, the North American Aerospace Defense Command (NORAD) initiated the creation of a comprehensive database known as the Space Object Catalog. This catalog aimed to document all recorded rocket launches and objects that entered orbit, including satellites, protective shields, and upper stages of launch vehicles. Subsequently, NASA released modified versions of this database in a format known as two-line element sets. Starting in the early 1980s, the CelesTrak bulletin board system also made these element sets available to the public.

During the 1980s, NASA and various other U.S. organizations endeavored to control the proliferation of space debris. One approach that was experimented with involved McDonnell Douglas in 1981, specifically in the case of the Delta launch vehicle. They designed the booster to move away from its payload and release any remaining propellant from its tanks. This innovation addressed one source of pressure build-up within the tanks, which had previously resulted in explosions and the creation of additional orbital debris. However, the adoption of such measures was relatively slow among

other countries, and the problem of space debris continued to worsen during the 1980s, particularly due to numerous launches conducted by the Soviet Union.

Subsequent to these developments, a series of studies were conducted by NASA, NORAD, and other relevant organizations, aimed at gaining a deeper understanding of the orbital environment. In these studies, the estimated number of objects falling within the critical mass zone was consistently revised upwards. For example, in 1981 (around the time of Schefter's article), it was estimated that there were approximately 5,000 objects in this zone. However, the deployment of new detectors in the Ground-based Electro-Optical Deep Space Surveillance system led to the discovery of additional objects.As we moved into the late 1990s, it was believed that most of the 28,000 objects launched into orbit had already re-entered the Earth's atmosphere, leaving around 8,500 objects still in orbit. By 2005, this estimate was adjusted upward to 13,000 objects, and a 2006 study increased the count to 19,000 due to an antisatellite (ASAT) test and a satellite collision. In 2011, NASA reported that they were tracking 22,000 objects in space.In 2006, a NASA model projected that if no new launches occurred, the space environment would maintain the then-known population until approximately 2055, after which it would naturally increase. Richard Crowther from Britain's Defence Evaluation and Research Agency, in 2002, anticipated that the cascade effect of space debris would likely commence around 2015. The National Academy of Sciences, summarizing expert opinions, pointed out a consensus that two specific regions within low Earth orbit (LEO) – the altitude bands of 900 to 1,000 kilometers (620 miles) and 1,500 kilometers (930 miles) – had already surpassed critical density levels

During the 2009 European Air and Space Conference, Hugh Lewis, a researcher from the University of Southampton, made a forecast that the threat posed by space debris would increase by 50 percent over the next decade and quadruple over the next 50 years. At that time, in 2009, there were more than 13,000 close encounters with space debris being tracked on a weekly basis. As of January 2019, it was estimated that there were over 128 million pieces of debris smaller than 1 centimeter (0.39 inches) in size, along with approximately 900,000 pieces ranging from 1 to 10 centimeters in size. The count of larger debris, defined as objects that are 10 centimeters across or larger, stood at 34,000 in 2019 and had increased to at least 37,000 by June 2023. It's worth noting that the technical measurement threshold for tracking debris is around 3 millimeters (0.12 inches)

In orbits situated close to Earth, specifically those at altitudes less than 2,000 kilometers (approximately 1,200 miles), collectively known as low-Earth orbit (LEO), there have traditionally been relatively few "universal orbits." These universal orbits are characterized by a significant number of spacecraft occupying specific rings within the orbit. This stands in contrast to geostationary orbit (GEO), which is a single orbital path commonly used by over 500 satellites. However, this began to change in 2019, as several companies embarked on the deployment of early-stage satellite internet constellations. These constellations consist of multiple universal orbits in LEO, with each orbital plane hosting 30 to 50 satellites. Traditionally, the most densely populated LEO orbits have been occupied by a variety of sun-synchronous satellites. These satellites maintain a consistent angle between their orbital plane and the Sun, facilitating Earth observation by ensuring a uniform sun angle and lighting. It's important to note that sun-synchronous orbits are polar, meaning they pass over Earth's polar regions. LEO satellites in general orbit in numerous planes and complete their orbits multiple times each day, leading to frequent close encounters between objects. As a result, the density of satellites, both operational and derelict, is considerably higher in LEO.

Orbital paths of objects in space are influenced by gravitational forces, which can vary due to irregularities in the Earth's gravitational field resulting from differences in the planet's density. In low Earth orbit (LEO), where these variations are especially noticeable, collisions between space objects can happen from any direction. Typically, collisions in LEO occur at an average speed of 10 kilometers per second (about 6.2 miles per second), but they can reach even higher speeds, exceeding 14 kilometers per second (approximately 8.7 miles per second) due to variations in orbital eccentricity. An illustrative example of this occurred during the 2009 satellite collision, where two satellites collided at a closing speed of 11.7 kilometers per second (equivalent to about 26,000 miles per hour). This collision resulted in the creation of more than 2,000 large debris fragments. These fragments then cross the paths of numerous other orbits, significantly elevating the risk of further collisions with space debris.

Kessler Syndrome

There is a theory suggesting that if a sufficiently large collision were to occur among spacecraft, it could potentially trigger a cascade effect, or even render certain low Earth orbits effectively unusable for long-term satellite deployment. This phenomenon is known as the Kessler syndrome. The theoretical scenario involves a runaway chain reaction of collisions that could exponentially increase the number and density of space debris in low-Earth orbit, and this is believed to occur once a certain critical density is reached.

Crewed space missions are typically conducted at altitudes of 400 kilometers (about 250 miles) and below, where atmospheric drag plays a role in gradually clearing regions of space debris. It's important to note that the upper atmosphere is not a constant density at any specific orbital altitude; it experiences variations due to atmospheric tides and undergoes expansions or contractions over extended periods due to space weather. These longer-term effects can enhance atmospheric drag at lower altitudes, and the expansion of the upper atmosphere during the 1990s contributed to a reduction in debris density. Additionally, a decrease in launches by Russia played a part in this

reduction, as most of their launches occurred during the 1970s and 1980s. At higher altitudes, where atmospheric drag is less significant, orbital decay takes a much longer time. Gradual processes like slight atmospheric drag, lunar perturbations, Earth's gravity perturbations, solar wind, and solar radiation pressure can gradually lower the altitude of space debris, causing it to eventually decay. However, at very high altitudes, this decay process can take centuries. While high-altitude orbits are less commonly used than those in low Earth orbit (LEO), the accumulation of debris toward the critical threshold is faster in these higher orbits.

Many communication satellites are positioned in geostationary orbits (GEO), where they cluster over specific target areas and share the same orbital path. While relative velocities between objects in GEO are relatively low, when a satellite becomes non-operational, like the case of Telstar 401, it transitions into a geosynchronous orbit. During this transition, its orbital inclination increases by about 0.8 degrees, and its speed escalates by approximately 160 kilometers per hour (99 miles per hour) annually. The impact velocity when two objects collide in GEO reaches a peak of about 1.5 kilometers per second (0.93 miles per second). Orbital perturbations cause the inoperative satellite to experience a shift in its longitudinal position and precession of its orbital plane. Close encounters, with distances as small as 50 meters, are estimated to occur approximately once a year. While the collision debris poses a lower short-term risk compared to collisions in low Earth orbit (LEO), the inoperative satellite is likely to become non-functional as a result of the collision. Larger objects, such as solar-power satellites, are particularly susceptible to such collisions. Although the International Telecommunication Union (ITU) now requires evidence that a satellite can be maneuvered out of its orbital slot at the end of its operational life, studies suggest that this requirement may not be sufficient. Since GEO orbits are too distant to accurately monitor objects smaller than 1 meter (about 3 feet 3 inches) in size, the precise nature of this problem remains unclear. One potential solution is to relocate satellites to unoccupied positions in GEO, which would necessitate less maneuvering and make future motion prediction more feasible. However, satellites or boosters in other orbits, especially those stranded in geostationary transfer orbits, present an additional concern due to their typically high crossing velocities

In spite of ongoing efforts to minimize the risk, incidents of spacecraft collisions have indeed occurred. For instance, the European Space Agency's telecommunications satellite, Olympus-1, experienced a collision with a meteoroid on August 11, 1993. Eventually, it was maneuvered into a

designated graveyard orbit. Similarly, on March 29, 2006, the Russian Express-AM11 communications satellite was struck by an unidentified object, rendering it non-functional. Engineers had sufficient time to reposition the satellite into a graveyard orbit before losing contact with it. In 1958, the United States launched Vanguard I into a medium Earth orbit (MEO). As of October 2009, both Vanguard I itself, the upper stage of its launch rocket, and a related piece of debris represent the oldest surviving artificial space objects still in orbit. These objects are expected to remain in space until after the year 2250.As of May 2022, the Union of Concerned Scientists reported a total of 5,465 operational satellites out of a known population of 27,000 tracked orbital debris objects monitored by NORAD. Periodically, satellites are left in orbit when they reach the end of their useful life. Many countries require satellites to undergo a process called passivation at the conclusion of their missions. During passivation, satellites are either boosted into a higher, designated "graveyard" orbit or placed into a lower, short-term orbit. However, satellites that have been properly relocated to a higher orbit still carry an eight-percent probability of experiencing punctures and coolant releases over a 50-year timeframe. This released coolant freezes into solid sodium-potassium alloy droplets, contributing to the creation of additional space debris.

Even with the implementation of passivation measures, or before they became standardized, numerous satellites and rocket bodies have experienced explosions or breakups while in orbit. For instance, in February 2015, the USAF Defense Meteorological Satellite Program Flight 13 (DMSP-F13) exploded in space, generating at least 149 debris fragments that were expected to linger in orbit for decades. Later in the same year, NOAA-16, which had been decommissioned following an anomaly in June 2014, disintegrated into at least 275 pieces while in orbit. For older satellite programs like the Soviet-era Meteor 2 and Kosmos satellites, design flaws led to multiple breakups – at least 68 by 1994 – after they were retired from service, contributing to further space debris. In addition to the unintentional creation of debris, there have been deliberate actions to generate space debris. This includes the intentional destruction of satellites, often as tests of anti-satellite or anti-ballistic missile technology, or to prevent sensitive satellite technology from being examined by foreign powers. The United States has conducted more than 30 anti-satellite weapons tests (ASATs), the Soviet Union/Russia has conducted at least 27, China has conducted 10, and India has conducted at least one. Recent ASAT events include China intercepting FY-1C, Russian trials of its PL-19 Nudol system, and the United States intercepting USA-193.

Space debris encompasses various items, including a glove lost by astronaut Ed White during the first American spacewalk (EVA), a camera dropped by Michael Collins near Gemini 10, a thermal blanket released during STS-88, garbage bags discarded by Soviet cosmonauts during the 15-year mission of the Mir space station, a wrench, and a toothbrush. During an EVA on STS-116, Sunita Williams lost a camera. In an EVA conducted during STS-120 to reinforce a torn solar panel, a pair of pliers was misplaced, and during an STS-126 EVA, HeidemarieStefanyshyn-Piper lost a briefcase-sized tool bag. Rocket upper stages that end up in orbit represent a significant source of space debris. In characterizing the space debris problem, it was discovered that many instances of debris were linked to rocket upper stages, such as the Inertial Upper Stage, that entered orbit and subsequently disintegrated due to the decomposition of unvented unburned fuel. The earliest such occurrence was associated with the launch of the Transit-4a satellite in 1961. Approximately two hours after insertion, the Ablestar upper stage exploded. Even intact boosters that remain undamaged can pose a problem, as exemplified by a notable impact event involving an Ariane booster.

Invisible peril: Space debris

One of the most pressing concerns regarding space pollution is the proliferation of space debris, also known as space junk. Space debris includes defunct satellites, discarded rocket stages, fragments from previous collisions, and even tiny pieces of paint and dust. These objects, often traveling at incredibly high velocities, pose a significant threat to active satellites, spacecraft, and astronauts in orbit.The origins of space debris can be traced back to human activities in space. Since the launch of the first artificial satellite, Sputnik 1, in 1957, thousands of satellites have been sent into orbit. Additionally, the stages of rockets used to deliver payloads into space are often left to orbit Earth after their mission is complete. Over time, these defunct satellites and rocket stages break apart, creating an ever-increasing cloud of debris that encircles our planet.

The consequences of space debris are far-reaching. Collisions with even small fragments can damage or destroy operational satellites, disrupt communication networks, and jeopardize the safety of astronauts aboard the International Space Station (ISS). Furthermore, as the amount of debris continues to grow, the risk of a cascading effect known as the Kessler syndrome, where collisions generate more debris, becomes increasingly likely. Such a scenario could make certain orbits unusable for centuries, hindering future space exploration efforts.

Birth of space junk

This implies that even objects as small as a pea can turn into perilous projectiles while orbiting the Earth. This was demonstrated in 2016 when a tiny speck of paint collided with a window on the International Space Station, creating a quarter-inch dent in the glass (fortunately, the window remained intact). Space debris often descends to Earth. On average, approximately 200 to 400 pieces of monitored space junk re-enter Earth's atmosphere annually, according to the National Oceanic and Atmospheric Administration. The majority of this descending debris is small enough to completely incinerate in the atmosphere, never reaching the Earth's surface. Larger objects that can endure the descent, such as satellites, typically land in the ocean, although exceptions occur. In August 2022, a charred, spear-shaped fragment of a SpaceX Crew Dragon spacecraft descended through the atmosphere and landed on a sheep farm in Australia

The 10 foot seared spike from a SpaceX vehicle was found standing upright in a sheep farm in Australia. (Image credit: Brad Tucker)

Space junk incidents

On February 10, 2009, a decommissioned Russian satellite collided with a functioning U.S. Iridium commercial satellite, leading to the destruction of both spacecraft and introducing more than 2,300 traceable pieces of space debris into the orbital zone, according to NASA's findings. In March 2021, a portion of a Russian rocket collided with and incapacitated an operational Chinese military satellite. In June 2021, a small, unidentified fragment of space debris struck the robotic arm of the International Space Station, causing damage but not rendering it non-functional. These occurrences are on the rise due to the increasing accumulation of space debris in orbit each year.

Light pollution from space

Light pollution represents another kind of contamination that reaches into the celestial domain. This occurs when human-made light sources on Earth emit an excessive amount of light, which scatters into the night sky, obscuring our view of stars and celestial objects. While it may not have an immediate impact as severe as space debris, it carries substantial cultural, scientific, and environmental implications.Urban areas are the primary contributors to light pollution on our planet, and their adverse effects on our capacity to observe and explore the universe from the Earth's surface are well-documented. Nevertheless, the consequences of light pollution also extend into space. The glare caused by artificial Earthly lights can disrupt astronomical observations conducted by space-based telescopes and observatories, thereby limiting our comprehension of the cosmos.

Space mining conundrum

The pursuit of responsible space exploration necessitates international collaboration and the formulation of clear guidelines and regulations. With the increasing involvement of numerous countries and private enterprises in the space industry, responsible practices become crucial. Taking measures to reduce space debris, minimize light pollution, and implement sustainable space mining practices are fundamental steps toward safeguarding the long-term well-being of the space environment. In summary, space pollution is an emerging challenge that requires our attention and thoughtful intervention. Whether it's managing space debris, reducing light pollution, or responsibly exploring extraterrestrial resources, we must make every effort to protect the final frontier as we venture deeper into the cosmos. Failing to do so not only endangers the future of space exploration but also affects our view of the universe and the delicate ecosystems of our home planet, Earth.

The silent threat above: Space junk

High in the vast expanse of space, an invisible danger looms—a hazard that has been accumulating for decades, a testament to humanity's pursuit of the stars and the consequences of our spacefaring endeavors. This chapter delves into the realm of space debris, the growing cloud of discarded objects orbiting our planet, and the challenges it presents to our ongoing exploration of space.

Peril of space junk collisions

The real menace posed by space debris is the potential for collisions. Objects hurtling through space at speeds of up to 17,500 miles per hour (28,000 kilometers per hour) mean that even a small fragment of debris can cause catastrophic damage. Satellites worth millions or billions of dollars are constantly at risk of being rendered nonfunctional, and the safety of astronauts aboard the International Space Station (ISS) is perpetually in jeopardy. Collisions can further exacerbate the problem, leading to a cascade of crashes known as the Kessler syndrome. This scenario, proposed by NASA scientist Donald J. Kessler in 1978, envisions a chain reaction of collisions that generates so much debris that certain orbital regions become nearly impassable for future generations.

Cleaning up the space debris

Addressing the issue of space debris is a formidable challenge. Various strategies have been suggested and, in some cases, put into practice:

Debris Tracking: Space agencies worldwide continually monitor and catalog objects in orbit, providing data to help spacecraft avoid potential collisions.

Active Removal: Concepts for active debris removal involve sending specialized spacecraft to capture and deorbit defunct satellites and other large pieces of debris.

Passive Deorbit Technologies: Designing satellites with built-in technologies that facilitate their natural deorbiting upon mission completion, ensuring they burn up in Earth's atmosphere.

International Cooperation: Spacefaring nations must collaborate to establish guidelines and regulations for responsible space operations, including debris mitigation practices.

Environmental Concerns

The continuous practice of discarding space debris on Earth, such as in locations like the spacecraft cemetery, has raised environmental concerns. Since 1971, 273 spacecraft and satellites have been deliberately directed to a remote spot in the ocean known as Point Nemo, including the sizable Mir Space Station (weighing 142 tonnes), with plans to dispose of the International Space Station in 2024 (weighing 240 tonnes). Alarming levels of microplastic particles have been discovered in the water at this site, indicating significant pollution. The growing issue of orbital debris has been likened to the environmental concept of "sacrifice zones" on Earth, which are regions marked by severe environmental degradation.

Moreover, rocket launches, which have occurred at more than three hundred launch sites worldwide since the 1960s, impact local and global environments through the construction of launch infrastructure, exposure to toxic residues, and the dispersion of pollutants. Rockets are the primary source of direct human-made emissions into the stratosphere, releasing substances that deplete ozone, such as nitrous oxide, hydrogen chloride, and aluminum oxide. Each launch results in the showering of toxic substances over a concentrated area within a kilometer, leading to local problems like acid rain, plant damage, fish die-offs, and failed seed germination. Research has even revealed the presence of toxic trace elements in wildlife near launch sites. Due to a lack of resources to maintain safe and non-toxic environments, these areas become sacrifice zones and zones of waste. Despite their remote locations making them suitable for rocket launches, they remain environmentally compromised.

The problem of orbital debris, stemming from increased human activities in outer space, is essentially a form of physical pollution that is causing congestion in space, endangering the operations of nations and corporations. However, the impacts of orbital debris extend beyond these entities and affect the general population. This includes the potential hazard of debris re-entering the Earth's atmosphere and causing harm, as well as the disruption of the night sky due to light pollution. When we consider the concept of environmental justice, as defined by the United States Environmental Protection Agency, it becomes apparent that space, much like terrestrial environments, can be subject to issues of environmental justice.

Environmental injustice involves protecting the rights of those who have suffered harm or intrusion from more powerful actors. In the context of the space industry, major space organizations and nations are the powerful actors, while the public represents those who may be affected. Recent research

indicates that space exploration raises significant questions related to environmental justice. The geopolitics of Earth and outer space are interconnected, particularly in terms of privilege and sacrifice. These aspects are also linked within the framework of environmental justice, including concerns such as emissions from space launches, the placement of space-related infrastructure, and, as discussed in this case, the issue of orbital debris.

Some argue that, in addition to considering space as an environment, it should also be regarded as a global commons. This perspective implies that space resources should be managed for the benefit of all, preventing potential injustices. Viewing outer space as a global commons poses challenges in legal definitions, as the concept of global commons is socially constructed. Various space treaties contain phrases emphasizing the use of space "for the benefit of all people" and as "the province of all mankind." However, these treaties lack sufficient frameworks for resource management and issue resolution.

Future of space debris

As our satellite launches and space exploration activities continue, the problem of space debris will persist and possibly intensify. It is our responsibility to act prudently, develop new technologies, and foster international collaboration to mitigate this threat. Space is a finite resource, and ensuring its long-term sustainability for scientific research and commercial endeavors necessitates the effective management of space debris.

In conclusion, space debris presents a multifaceted and growing challenge that impacts not only our ongoing space missions but also the future of space exploration. Our ability to navigate the increasingly crowded orbital environment and safeguard valuable assets in space hinges on our collective efforts to manage and alleviate the risks posed by space debris. The invisible threat from above serves as a reminder that, despite the vastness of the universe, our responsibilities to it are very real.

Conclusion

In navigating the cosmos and unraveling the growing issue of space pollution, it becomes abundantly clear that the challenges posed by human activities beyond our planet are not confined to the vastness of outer space. Space pollution, in the form of orbital debris and its environmental consequences, serves as a sobering reminder that our actions in space have repercussions that reverberate back to Earth.

As we look to the heavens with dreams of exploring new frontiers and expanding our understanding of the universe, we must do so with a heightened sense of responsibility. The accumulation of space debris, deliberate disposal in remote locations like Point Nemo, and the environmental impacts of rocket launches all demand our attention. This is not a problem that solely concerns nations and space agencies; it affects us all. The dangers of space debris re-entering our atmosphere, light pollution disrupting our view of the night sky, and the environmental justice issues associated with space exploration have global implications.

Moreover, the concept of space as a global commons underscores the importance of collaborative efforts in managing and mitigating space pollution. International cooperation, robust space debris mitigation strategies, and the development of sustainable space practices are essential for ensuring the long-term viability of space exploration and satellite operations.

In conclusion, the issue of space pollution is a multifaceted and pressing concern. As we continue our journey into the cosmos, it is our collective duty to safeguard the celestial environment, protect our shared global commons, and ensure that the promise of space exploration benefits not just a select few, but all of humanity. The cosmos may be vast, but our commitment to its preservation and responsible exploration is very much grounded in our terrestrial reality.

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Demystifying Midas touch by bacteria – Gold Biomineralization in *Cupriavidus metallidurans*

Dr. A. Kiruthiga*
Associate Professor in Microbiology,
Madha Dental College and Hospital
(Affiliated to The T.N. Dr. M.G.R. Medical University),
Kundrathur,
Chennai – 600 069
Email: *kiruthigaalexander@gmail.com*

Dr. R. Deepa
Associate Professor in Biochemistry,
Madha Dental College and Hospital
(Affiliated to The T.N. Dr. M.G.R. Medical University),
Kundrathur,
Chennai – 600 069
Email: *deepanish1981@gmail.com*

Abstract

Microbes play a significant role in the biogeochemical cycling of metals resulting in the formation of minerals. Microbial-mediated metal cycling involves processes like solubilization, transportation and reprecipitation of the metals. This may result in the development of genetic and proteomic responses for the regulation of metal homeostasis. The process of microbial weathering leads to gold mobilization liberating gold elements entombed within the minerals and mediating gold solubilization through complex oxidation reactions. Gram-negative, Metallophillic β proteomic bacterium such as *Cupriavidus metallidurans* (formerly called *Ralstonia metallidurans*) brings about the detoxification of gold complex resulting in the transformation of metallic gold. Hence gold biomineralization using these bacterial communities would pave the way for future research

Keywords: Microbes, biogeochemical cycling, biomineralization, *Cupriavidus metallidurans*

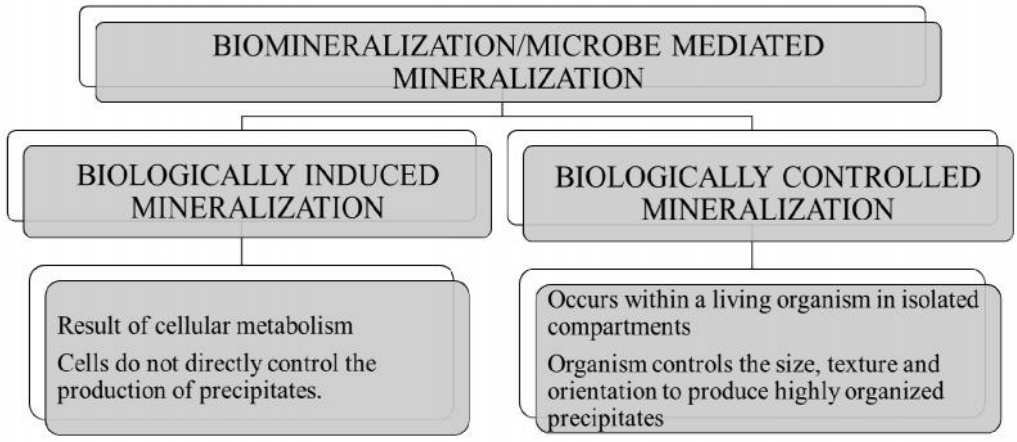
Introduction

Inorganic formation of certain minerals is likely to be synthesized by certain organisms in the environment. The final configuration of the mineral may either deviate from the initial form or may be substituted by another mineral during the organism's development [1]. Biomineral not only refers to minerals produced by an organism but also denotes the mineral substances [2] with the complexity of organic substances having physicochemical characteristics like size, shape, crystal morphology, isotopic forms and compositions of trace elements dissimilar to the synthesized inorganic counterpart [3].Biomineralization may be defined as a process in which minerals are produced by living organisms. Examples of biominerals include silicates, calcium carbonates and phosphates synthesized in algae, diatoms, invertebrates and vertebrates.

Classification of synthesis of minerals by prokaryotes:

Minerals synthesized by prokaryotes can be widely classified as Biologically induced mineralization (BIM) and Biologically controlled mineralization (BCM) [4,5]. (Fig.1: Synthesis of Minerals by Prokaryotes, Qin et al, 2020)

Fig.1. SYNTHESIS OF MINERALS BY PROKARYOTES



Biological induced mineralization:

Biologically induced mineralization involves the metabolism or byproducts of metabolism succeeding biochemical reactions of the organism resulting in the nucleation and extracellular formation of minerals [7]. Familiar

example of biologically induced mineralization is the Ureolytic pathway of biomineralization where the soil bacterium *Sporosarcina pasteurii* bring about urea hydrolysis resulting in increased pH levels and produces calcium carbonate in the presence of calcium [8].

Biological controlled mineralization:

Biologically controlled mineralization can also be called organic-matrix-mediated mineralization [1] and boundary-organized biomineralization [9]. Well-characterized BCM example is the formation of magnetosomes by magnetotactic prokaryotes producing crystalline magnetic substances with a comparatively confined role [10]. Magnetotactic bacteria synthesize two types of biominerals i.e., magnetite crystals (iron oxide) and greigite crystals (iron sulfide) [11].

Table 1: Differences between Biologically induced mineralization and Biological controlled mineralization

Characteristics of Biomineralization	Biologically induced mineralization	Biological controlled mineralization
Degree of biological control over the mineral formation	Uncontrolled sequence of metabolic activities over the mineral formation	Widespread control over the mineral formation
Structure /configuration of the formed mineral	Well-defined mineral configuration	Heterogenous mineral configuration
Variation	Minor size variations	Large size variations
Crystal morphology	Species-specific crystal habits	Poorly defined crystal morphology

The chemistry of biomineralization is highly influenced by three prime factors;

i) Chemical composition, biochemistry and crystallography of thebiological substance,
ii) Development of the *in vitro* model system,

iii) Introduction of newer techniques regulating the process resulting in novel composites of organic-inorganic substances [13].

Microbes are considered significant in the biogeochemical cycling of metals like Magnesium (Mg), Sodium (Na), Cobalt (Co), Iron (Fe), Copper (Cu), Molybdenum (Mo), Zinc (Zn), Nickel (Ni), Vanadium (V) and Tungsten(W), as they are indispensable for their nutritional needs [14]. The active participation of bacteria in the process of biomineralization helps in the maintenance of neutral conditions on our planet [15,16]. Recent studies targeted the various methods of bacterial response towards the status of metal ions, the ways in which metal limitation inhibits bacterial growth, the bactericidal activity of intoxication of metals related to the interaction between a host and a pathogen [17].

Although the role of microbes is universally accepted in the mobility of metals and the formation of minerals, abiotic methods involve secondary gold (Au) formation [18]. Compared to other metals, gold (Au) is not common and they do not produce free ions in an aqueous solution [19]. Cupriavidus metallidurans formerly called Ralstonia metallidurans can withstand an environment with increased concentration of more than 20 heavy metals [20]. CH34 strain of this metallophillic β proteobacteria plays a predominant role in the biofilm on the natural form of gold (Au) biomineralization [21]

Fe (III)-reductase present in the bacterial and Archaeal members mediates precipitation and reduces Au-complex [22]. Transenvelope efflux exports Au(I) protects the periplasmic space of Gram-negative bacteria against redox stress [23]. Au(I) after entering the bacterial cytoplasm causes oxidative stress [24], Au(I) binds to either CueR/CupR controls gene expression of P-type ATPase enzymes and other enzymes mediating copper resistance [25]. Active participation of CopA, GoIT and CupA efflux pumps in certain Gram-negative bacteria are found to be involved in the activation of the Au complexes mediated by enzymes CueR, GolS and CupR can be evidenced in *Escherichia coli*, *Salmonella* and *Cupriavidus metallidurans* respectively [26,27,28]. Au ions are exported by GolT [29] while these ions inhibit CopA [28] and CupA [30].

Mechanism of biomineralization:

Au-regulated gene expression results in the energy-dependent reduction reaction precipitating the complex of Au (III) is evidenced in the CH 34 strain of metallophillic bacteria *Cupriavidus metallidurans*, This in turn results in the formation of biofilms and accumulation of Au (III) complex. Coupled with the

AU(I)-S complex, Au toxicity is enhanced followed by the induction of oxidative stress and resistance gene clusters towards metals. in metallophillic bacterium. Detoxification of Au occurs by the combined reactions of reduction, efflux and addition of methyl groups to Au complexes. This biomineralization process finally forms the Au(I)-C complex and Au nanoparticles [18].

Conclusion

Biominerals, though considered the principal constituents of biogeochemical cycles of metals occurring naturally, are also significant in the process of depolluting the environment [31]. Microbe-mediated biomineralization processes can be established technically as biosensors. Identification of such Au-specific genetic response can enable the biosensor technology of Au which can serve as a revolutionary marker in exploring the ways of efficient extraction of Au and processes of hydrometallurgy [18].

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Parasitic infestations of the Human Oral Cavity – Intruders unveiled

Dr. K. Padmavathy[1], Dr. A. Kiruthiga[2]* and Ms. A. Iswarya[3]

[1]Professor, Department of Microbiology, Research Laboratory for Oral and Systemic Health, Sree Balaji Dental College and Hospital, BIHER, Chennai. India

[2*]Associate Professor, Department of Microbiology, Madha Dental College and Hospital (Affiliated to the T.N. Dr. M.G.R. Medical University), Kundrathur, Chennai.

[3]Lecturer, Department of Microbiology, Madha Dental College and Hospital, (Affiliated to the T.N. Dr. M.G.R. Medical University), Kundrathur, Chennai.

Abstract

Infestations caused by parasites constitute different members of unicellular protozoans, unicellular eukaryotic organisms and multicellular helminths. Severe forms of parasitic infections occur in the gastrointestinal, cutaneous and various parts of the body with ingestion as the common route of transmission. The oral cavity serves as an abode for the colonization of various types of emerging parasites leading to the development of lesions.

Keywords: Parasitic infestation, Ingestion, Oral cavity, Lesions

Introduction

Parasitic infections in the oral cavity

The human oral cavity serves as the portal of entry for numerous parasites. Very few parasites thrive in occupying the oral cavity as their habitat. Parasitic members of the oral cavity include harmless commensals turning into opportunistic pathogens such as *Trichomonas tenax* [1] and *Entamoeba gingivalis* [2], while the serious pathogenic forms include *Naegleria fowleri* and *Acanthamoeba encephalitis* [3] causing meningoencephalitis following their way to the brain after entering through the oral cavity. These parasites may reach the nasal cavity and olfactory nerve finally resulting in the invasion of the brain [3-5].

Leishmania species are capable of causing serious oral infections following granulomatous defacements involving the oral and nasal cavities [6]. Leishmaniasis, a vector-borne parasitic infection characterized by chronic inflammatory disease, is caused by a protozoan flagellated parasite transmitted through insect vectors of the genera *Phlebotomus* or *Lutzomyia* species [7].

Migration of nematodes to the superficial tissues of the host following ingestion or after gaining entry through the skin where the activity of the parasite is usually limited to the cutaneous region. Migration of the nematodes to the oral cavity is uncommon but more commonly seen in the infestation caused by *Gongylonema pulchrum* [8].

Commonly encountered other oral helminthic infections include trichuriasis, filariasis, trichinosis, larva migrans, cysticercosis, echinococcosis and sparganosis [9].

Trichomoniasis:

Trichomonas tenax is an anaerobic protozoan possessing flagella and capable of living in a hypoxic environment. Though showing the absence of mitochondria, this parasite represents the ancestral member of eukaryotic origin [10]. The oral cavity of humans, as well as animals, are found to be the habitat of *Trichomonas tenax* may be encountered in parasitic periodontal diseases in disparity with the established pathogenic member of the same genus, *Trichomonas vaginalis* inhabits the human genital tract [11]. Apart from getting addressed as an oral commensal, the parasite *T. tenax* can also be present in various regions like lymph nodes, tonsils, glands present in the submaxillary region [12], bronchi and lungs [13]. The prevalence of this parasite in the human oral cavity was first reported by Mueller in 1773 [14].

Amoebiasis:

Entamoeba gingivalis, a eukaryotic protozoan parasite, colonizes the oral cavity of healthy humans. Occurrence of this protozoan member ranges from approximately 15% while an increased prevalence rate of about 70 – 80% is seen in the inflamed periodontal pockets of patients suffering from periodontitis [15]. *Entamoeba gingivalis* is the only species among the genera Entamoeba found to colonize the oral cavity of humans. Other species of Entamoeba inhabiting humans colonize in the lumen of the intestine include *Entamoeba histolytica*, a predominant parasite commonly encountered in the etiology of amoebiasis [16]. According to a study by Garcia et al, two subtypes of *Entamoeba gingivalis* ST1 and ST2-kamaktli subtypes are prevalent in the oral cavity of humans under various conditions [17].

Primary amoebic meningoencephalitis:

The Genus Naegleria, a eukaryotic free-living amoeba, is classified in the phylum Percolozoa under the family Vahlkampfiidae placed in the order Schizopyrenida with Class Heterolobosea [18]. Though 47 species of Naegleria remain identified to date, Pathogenic species of Naegleria include *N. australiensis, N.italica* and *N. fowleri* [19]. *N. australiensis* and *N.italica* are capable of infecting only laboratory animals while *N. fowleri* is considered medically significant as they are responsible for causing primary amoebic meningoencephalitis (PAM) *among* humans [19, 20]. This infection may be characterized as an acute fulminating infection which may become fatal after a week or 10 days followed by the entry of amoeba inside the body of the host [21]. This parasite, named after Malcolm Fowler following his first report of Primary amoebic encephalitis in Australia, is also popularly known as "brain-eating amoeba" [22]. This parasite derives its nutrition from bacteria and organic waste present in freshwater. The parasite completes its life cycle in three forms: cyst, trophozoite and flagellate form with their possible mode of transmission is through the nose via recreational activities such as deep inhalation of water contaminated with this parasite [23].

Acanthamoeba encephalitis:

Acanthamoeba species, an opportunistic parasite, is the etiological agent of Acanthamoeba keratitis (AK) and fatal granulomatous amoebic encephalitis (GAE) in the immunocompromised individuals [24]. Recent studies suggested the evidence of Acanthamoeba occurring in the oral cavity of immunocompromised individuals associated with heart transplantation [25], patients suffering from chronic kidney disease [26] and patients presenting with the pulmonary symptoms suspected of malignancy [27].

Oral leishmaniasis

Mucosal leishmaniasis in the oral cavity without cutaneous involvement is uncommon. Lesions caused by this parasite typically appear as an erythema, progress as an ulcer or plaque, further forming papules and exophytic nodules in the oral cavity with hard or soft palate and tongue as their commonest sites of infection. Other sites of oral leishmaniasis include the lip, uvula, gingiva, tonsils and retromolar areas [28].

Gongylonemiasis:

Gongylonema pulchrum causes Gongylonemiasis. Infections caused by Gongylonema are occasional among humans and are commonly encountered as zoonotic infections among domestic cattle and other

animals [8]. Animals feed on the insects and acquire the infection while accidental infection occurs among humans by ingestion of an intermediate host including beetles and cockroaches [29]. Adult worms of *Gongylonema* can parasitize humans for about 10 years and are responsible for causing local irritative symptoms in the oral cavity [30].

Oral helminthic infections:

Trichuriasis:

Trichuriasis, caused by *Trichuris trichiura, is* responsible for the mucosal lesions in the oral cavity. The mode of transmission may be due to the behavior of patients having a hand to mouth with the hands contaminated with the eggs of the parasite. Microscopic observation of the mucosal lesions in the lingual and labial region showed the incidence of the eggs of *T. trichiura* [31].

Filariasis:

Medically important filarial nematodes include *Wuchereria bancrofti*, *Brugia malayi*, *Loa loa* and *Onchocerca volvulus*. *Wuchereria bancrofti* accounts for the predominant filarial worm in India. The parasite gains entry inside the body of the host through the bite of mosquitoes. Clinical manifestations of filariasis infection show inflammation and enlargement of the lymph nodes resulting in elephantiasis of the legs, arm, scrotum and breast [32]. In young patients presented with microfilaremia, oral and perioral regions are occasionally involved, typically manifested with the oedematous swelling of the lips and interdental papillae [33].

Trichinosis:

Human Trichinosis, is an infection caused by *Trichinella spiralis*, predominantly seen among pork eaters. Ingestion of raw or undercooked pork containing the infective stage of larva or adult nematode is the potential mode of transmission [34]. According to a study by Cheung et al, Trichinosis does not induce the tumor without a carcinogen while the nematode can act as a co-carcinogen and bring about the pathogenesis of tongue carcinoma [35].

Larva migrans:

Larva migrans are characterized by pruriginous or serpiginous lesions caused by the migration of the nematode *Ancylostoma brazilinese* [36]. Lip infection by this parasitic nematode involving only the oral mucosa is called as “parasitic migratory stomatitis” [37].

Oral cysticercosis:

Cysticercus cellulosae, the larval stage of *Taenia solium* causes Cysticercosis with their possible mode of transmission through the faeco-oral route [38]. Examination of the oral cavity for cysticercosis shows the presence of a circumscribed nodule with an intact mucosa. Nodules appeared soft, showing bluish discoloration, painless and mobile, measuring 1 cm in diameter is evident from a case report of a 7-year-old Mexican girl whose major complaint was a lump in her mouth. Macroscopic examination of the mucocele showed the presence of a wedge-shaped mucosa while the microscopic observation revealed the presence of *Cysticercus cellulosae*, a larval form of *Taenia solium* [39].

Sparganosis:

Sparganosis in humans occurs by oral transmission or by plecocercoid larval penetration of the tapeworm belonging to the genera Spirometra [40]. Mode of transmission of Sparganosis includes i) Ingestion of the infected cyclops with procercoid stage, ii) Ingestion of plerocercoid in raw flesh of animals, iii) Contact with the flesh of infected vertebrates leading to the movement of Sparganum into tissues of humans [41].

Echinococcosis:

Echinococcosis parasitizing humans is caused by *Echinococcus granulosis* [42]. In India, this endemic disease is commonly seen in canines and bovines with an incidence rate between 1-200 per 100,000 people [43]. The term Hydatid is derived from the Greek word meaning “Drop of water” [44]. Hydatid cyst is predominantly encountered in the vital organs including the liver and lungs followed by the skin, kidney, spleen and muscles [45]. According to a study conducted by Alaparthi et al, an uncommon incidence of Intraoral hydatid cyst with a localized swelling caused by hydatid cyst was reported [46] and the localization of intra-oral hydatid cyst in the labial mucosa was documented by Banerjee *et al*, [47].

Conclusion

The emergence of new parasitic infections worldwide creates an alarming threat to public health. Studies targeting the new emerging parasites in the oral cavity of healthy and immunocompromised individuals should be acknowledged with the knowledge of their predominant symptoms which may be useful in the effective management of serious life-threatening parasitic infections.

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Insect pheromones: Chemistry and applications

[1]T. Poornakala, [2]S. Muniasamy, [3]T. Rajagopal, [4]G. Archunan, [1]P. Ponmanickam*

[1]Department of Zoology, [2]Department of Microbiology, Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi-626 124, Tamil Nadu, India.

[3]Department of Zoology, Thiagarajar College (Autonomous), Madurai 625 009, Tamil Nadu, India

[4]Research Dean, Marudupandiyar College, Thanjavur 613 403, Tamil Nadu, India

*Correspondence: *ponmanickam_ts228@anjaconline.org; pon_manick@yahoo.co.in*

Abstract

Animals communicate between and within species to exchange information. Chemical communication is one kind of adaptation in animals. Like all other animals, insects use their five senses to acquire information. Among these, the most common way of insect communication between organisms is chemical communication and it is broadly termed as "Semiochemicals". Pheromones and allelochemicals are the two groups that come under Semiochemicals. Hormones are chemicals that are released within the organisms, and it is secreted by endocrine glands (inside the body). Pheromones (intraspecific communication) are also like hormones but secreted by exocrine glands (outside the body), hence it is called 'Ectohormones'. Primer (delayed effect) and releaser pheromones (immediate response) are the two types of pheromones. Releaser pheromones are further divided into sex, alarm, aggregation, trail, and epideictic pheromones based on their function. Allelochemicals are used for interspecific communication and it is divided into Allomones, Synomones, Kairomones, and Apneumones. In this chapter, various types of insect pheromones, sensory systems, sources, chemical nature, hormonal regulation, and applications were discussed with suitable examples. Further, it is also compared with mammalian pheromone communication.

Key words: Semiochemicals, insect pheromones, sex pheromones, insect pest management

1.0 Introduction

Communication is especially significant for the survival of the organisms. No one animal survives independently and it is one of the adaptations. Insects communicate inter-specific and intra-specifically through a combination of mechanisms like chemical, tactile, acoustic, and visual means. "Cues" are called the language of insects. Cues are individual characteristics that are communicated by a producer and utilized by a receiver to decipher data about the producer (Padimi *et al*., 2023).

2.0 Chemical Communication

According to Padimi *et al*. (2023), there are eight major reasons (https://www.slideshare.net/AmmadAhmad10/communication-in-insects) for the insects to communicate and are listed below:

1. Identification of family members in the nest,

2. Finding a member of the opposite sex,

3. Help with courtship and mating,

4. Giving directions for the location of food,

5. Regulating spatial distribution of individuals, aggregation or dispersal; establishing and maintaining a territory,

6. Cautioning of risk and setting off an alert,

7. Communicating danger and risk and

8. Mimicry.

According to Dethier *et al.* (1960), these chemical communications serve six functions namely, 1.Locomotory stimulants, 2. Attractants, 3. Arrestants, 4. Repellants, 5.Feeding and ovipositional stimulants and 6.Deterrents.

3.0 Semiochemicals

The chemical which conveys information between two organisms are called 'semiochemicals' and are divided into two subcategories namely, Allelochemicals and Pheromones (Padimi *et al.*, 2023). Both terms are different as mentioned in the table-1.

Table-1: Contrast among allelochemicals and pheromones

Allelochemicals	Pheromones
Used in interspecific communication (between species).	Used in intraspecific communication (within species).
Emitted during biotic and abiotic stress.	Secreted as liquids from exocrine glands.
Further divided into allomones, synomone, kairomone and apneumone.	Further divided into Primer and Releaser pheromone.

4.0 Allelochemicals

Semiochemicals that are used in interspecific communication are called 'allelochemicals'. Allelochemicals are further divided depending on the costs and benefits to 'signaller' and 'receiver' (Nordlund, 1976) into Allomones, Synomones, Kairomones, and Apneumones.

1. Allomones: Animals of one species can emit signals that benefit themselves at the cost of the receiving species. Chemical signals used in such deceit or propaganda are termed 'allomones'. For example, Bolas spiders synthesize particular moth pheromones to lure male moths of those species into range for capture (Wyatt, 2003).
2. Synomones: Semiochemicals helping both signaller and recipient in mutualism. Predators likewise transmit synomones. In the mutualistic connection between the subterranean insect, *Pheidole bicornis*, and the plant *Piper cenocladum*, big quantities of nutritious plant-created food bodies show up just when *P.bicornis* is available. When other *Pheidole* species occupy the plant, no food bodies are delivered (Risch and Rickson, 1981).
3. Kairomones: If a predator uses the allelochemicals delivered by the prey and finds them, then, at that point, such chemicals can be named 'kairomones' (Brown *et al.* 1970). Kairomone advantages the beneficiary while allomones benefit the producer.
4. Apneumones: Emitted by a non-living source, causing a favorable behavioral or physiological reaction to a receiving organism, but harmful to other species that may be found either in or on the non-living material.

5.0 Pheromones

Similarly, pheromones are chemical cues/ chemical language produced by insects to communicate with conspecifics by which they evoke specific behaviors or responses in the receiver. They play a crucial role in various aspects of insect behavior, including mating, territory marking, and aggregation. Karlson and Butenandt(1959) have proposed the name "Pheromone" for the chemical compounds that enable members of the same species to communicate with each other. The term pheromone was derived from the Greek "pherein" (to carry) and "horman" (to excite, stimulate) (Regnier and Law, 1968) (https://projectdolittle.com/chemical-semantics/). The term "hormone" is different from pheromone as mentioned in the table-2.

Table-2: Difference between Hormones and Pheromones

S.No.	Hormones / Endohormones	Pheromones/ Ectohormones
1.	These are regulatory substances, that run through the blood and activate the cells to carry out their functions	Air-borne chemicals released by insects/animals, alter the behavior of recipient individuals of the same species.
2.	Works on the inside of the body of the releaser	Works on the outside of the body of the releaser
3.	There are many functions in the body	Have few functions in the body.
4.	Coordinate the individual secreting the hormone.	Integrate the population of the individual releasing the pheromone.
5.	Examples: Juvenile hormone, Ecdysone	Examples: sex pheromones, trail pheromones, alarm pheromones and aggregation pheromones.

6.0 Types of Insect Pheromones

The efficiency of pheromonal action depends on the following factors: i) the volatility of the compound, ii) its stability in air, iii) its rate of diffusion, iv) the olfactory efficiency of the receiver, and v) the speed of wind currents. Long-distance communication of a mile or more must be indicated by the use of stable compounds with high vapor pressures (Tembhare, 2016).

Pheromones are further divided into primer pheromones and releaser pheromones (As mentioned in Table 3). Pheromones have two ways of impacting the central nervous system (Wilson and Bossert, 1963). As in the case of releaser pheromones, one class of chemicals elicits an immediate behavioral response upon receiving. Primer pheromones, on the other hand, have a delayed effect on behavior. It influences the reproduction, development, and recognition of learning (Kost, 2008). Primer pheromones have an inhibitory effect on the growth of the population in social insects and sometimes an accelerating effect such as in dessert locusts, where the primer pheromone released by adult females accelerate the growth of both male and female nymphs to achieve synchronous growth within the species (Regnier and Law, 1968).

The classification of communication between insects is given below in the flow chart (Fig. 1) (Padimi *et al.*, 2023).

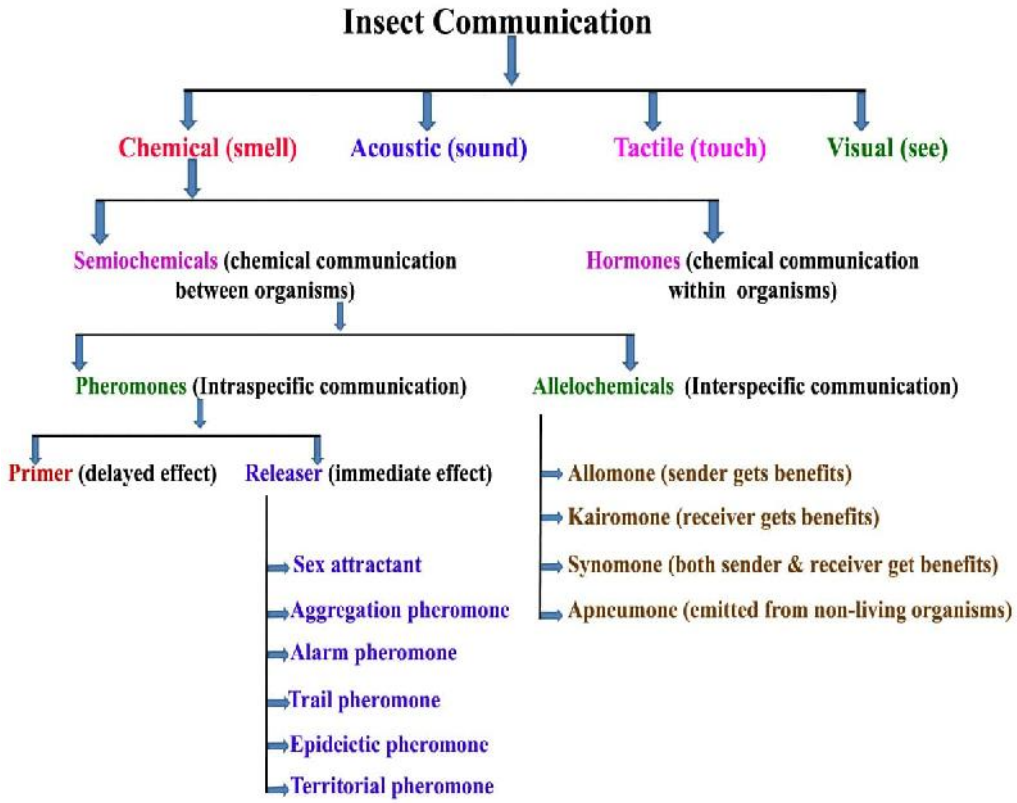

Fig. 1. Classification of chemical communication

Table-3: Difference between Primer Pheromone and Releaser Pheromone

S.No.	Primer Pheromone	Releaser Pheromone
1.	Elicits delayed effect	Elicits an immediate behavioural response
2.	Alter the physiology and behaviour	Alter the behaviour
3.	Prolonged effect	Short-term effect

Queen Pheromones: It is a classical example of a primer pheromone. In social insects, the queen produces pheromones that regulate the behavior and development of other colony members. The queen substance is composed of 9-oxodecenoic acid and 9 hydroxydecenoic acid (Tembhare, 2016). Another example of primer pheromone is 9-keto-1-decenoic acid which plays an important role in control of behavior in honeybee society. It acts as a sex pheromone by a virgin queen to lure the drones during mating flight and after mating the same substance is used for inhibitory behavior.

Releaser Pheromones: Releaser pheromone consists of the following types:

1. Sex Pheromones:

The first sex pheromone identified was (10E,12Z)-10,12-hexadecadienol or bombykol, the sex pheromone of the silkworm moth, *Bombyx mori* (Butenandt *et al.*, 1959). They are delivered by one sex just and trigger the behavior of the other sex individual for successful mating. Sex pheromones are generally released by females but rarely delivered by male individuals. In over 150 species of insects, females have been found to release sex pheromones and about 50 species of males produce (http://eagri.org/eagri50/ENTO232/lec21.pdf). Aphrodisiacs are substances that aid in courtship of the insects after the two sexes are brought together (https://www.studocu.com/in/document/dr-ys-parmar-university-of-horticulture-and-forestry/insect-anatomy/pheromones/43293983; https://www.scribd.com/document/82858322/20-Principos-aplicados - entomologia-1#). In many cases males produce aphrodisiacs. Major differences between male and female-produced pheromones are listed below (Table 4):

Table-4: Differences between female and male sex pheromone

S.No	Properties	Female Sex Pheromone	Male Sex Pheromone
1.	Range	Acts at a long range. Attracts males from long distance	Acts at a short distance
2.	Role of other Stimuli	Play less role	Visual and auduitory stimuli play major role
3.	Action elicited in the other sex	Atrracts and excites males to Copulate	Lowers females resistance to Mating
4.	Importance in IPM	More important	Less important

2. Alarm Pheromones: These are transmitted when an insect is in danger, making nearby conspecifics aware of the presence of risk. Eg. Ant discharges alarm pheromones from their mandibular organs when attacked and is also released as a responsive attack by aphids against natural enemies. But in some cases, these alarm pheromones may cause aggressive responses in social insects like bees and leaf-cutting ants (Ginzel, 2010).

3. Aggregation Pheromones: It is meant for aggregation of particular insects of the same species in a specific area to share food or shelter. Eg. Beetles, release aggregation pheromones from specialized glands in their cuticle. Aggregation pheromones are responsible for delivering signals that promote intraspecific group formation and mating at a food source (Tinzaara *et al.,* 2002; Kumar and Shahid, 2020).

4. Anti-aggregation Pheromones: It is opposite to aggregation pheromone. It causes the dispersal and disaggregation of individuals. Such dispersal works best under resource-limited environments to encourage the maintenance of optimum spacing (Mishra, 2020).

5. Trail Pheromones: It is mostly utilized by social insects, particularly ants and termites. The different individuals from the same species can follow the aroma trail to explore them from the home to a food source and back (Mishra *et al.,* 2020; Padimi *et al.,* 2023). Ants use this pheromone to mark the routes

of food sources from the home. These are typically secreted from the hindgut or specialized glands located near the stinger. Eg. Methyl-4-methyl pyrrole 2-carboxylate is the highly active substance produced by the leaf-cutting ant, *Atta texana* (Tumlinson *et al.,* 1971).

6. Territorial Pheromones: These are released to establish and defend territories. In some social insects like bees, they are produced by specialized scent glands associated with the cuticle (Padimi *et al.,* 2023).

7. Epideictic Pheromones: These are synthetic versions of sex pheromones that are used in pest management. They interfere with mating by saturating the environment with the scent, confusing male insects and preventing them from locating females. Eg. During the ovipositor dragging process after egg laying, a kind of compound called oviposition-deterring fruit-marking pheromones is deposited by females of several fruit fly species (eg. Diptera: Tephritidae), eg., Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Prokopy *et al.*,1978; Kumar and Shahid, 2020).

7.0 Chemical Nature of Pheromones

Pheromones are otherwise known as "ectohormones" (Mathur, 2010) and are produced in small amounts like hormones and serve as chemical messengers. The specific glands or structures involved in pheromone production can differ between insect species. These glands are often located in various parts of the insect's body, including the abdomen, thorax, and head, and are adapted to release volatile compounds that can travel through the air and be detected by other insects (Rizvi *et al.,* 2021). Lepidopteran insects have scent glands on their wings and in some scales, it may occur on the legs or abdomen. For instance, In *Ephestia ruaniella,* the male has a dorsolateral tuft of androconia on each side of the eighth abdominal segment whereas the males of *Amauris niavius* have a small scent patch on each side of the hind wings (Mathur, 2010). In Hymenoptera, *Apis* has two important pheromone-producing glands, they are, mandibular glands in the head and the Nassanoffis gland in the abdomen whereas Ants have additional Poison glands and Dufour's glands other than mandibular glands.

Insect pheromones are secreted in extremely low amounts, i.e., nanograms to micrograms per unit of time. Moreover, the amount of pheromones secreted varies with the insect species (Mishra *et al*., 2020). Pheromone release depends on certain factors like circadian rhythm, temperature, presence of food sources, and age of the insects (Mishra *et al.,* 2020). The molecular weight of insect pheromones varies widely depending on

the specific chemical compound. Insect pheromones can range from simple compounds with low molecular weights to more complex molecules with higher molecular weights. The molecular weight of a pheromone is determined by the specific arrangement of atoms and functional groups in its chemical structure. The molecular weights of pheromones usually do not exceed 250 g/mol (1 g/mol=1 Dalton) (Table 5) (Regnier and Law, 1968). Here are a few examples of insect pheromones along with their molecular weights:

Table-5: Molecular weight of pheromones

S.No.	Insect	Role of Pheromone	Compound	Molecular weight	Reference
1.	Almond Moth (*Ephestia cautella)*	Sex Pheromone	E,Z-Isomer of 9,12-Tetradecadienyl acetate (ZETA)	260 g/mol	Ding *et al.*,2022
2.	Ants (*Camponotus modoc)*	Trail Pheromone	(Z)-11-Hexadecenal	238 g/mol	Chalissery *et al.*, 2019
3.	Pea Aphids (*Acyrthosiphon pisum)*	Alarm Pheromone	(E)-β-Farnesene (EBF)	204 g/mol	Zhang *et al.*, 2017
4.	Bark beetles	Aggregation Pheromone	(Z)-9-Tetradecenol	212 g/mol	Rizvi *et al.*, 2021
5.	Fruit flies	Sex Pheromone	Citral	152 g/mol	NCBI, 2023
6.	Bees (*Apis mellifera)*	Alarm Pheromone	4-Methyl-3-heptanol	116 g/mol	Wang *et al.*, 2019

8.0 Sources of insect pheromones:

Both secretions and excretions are the sources of pheromones in insects. In insects, the research on the identification of sources is scanty. Very few reports are available to show the feces as a source of pheromones. Similarly, the scent sources of all the insect orders were not ruled out. Here only a few reports are mentioned.

Feces

Feces are one of the important sources of pheromones. Odors emanating from feces attract the same species as well as predators and parasites. For instance, pheromones from adult *Drosophila* attract conspecifics and accelerate feeding (Keesey *et al*., 2016). In locusts, a gregarizing pheromone was identified in the feces (Gillett and Phillips, 1977; Obeng-Ofori *et al*., 1994). Expanding population size causes the accumulation of gregarizing pheromones in feces, which alters the body size, color, and behavior of the locust, i.e. solitary stage to gregarious stage (Heifetz *et al*., 1996).

Exocrine glands

The exocrine glands are otherwise known as scent glands. It releases the secretory products to the outside of the body. These glands can be present on any part of the body – head, thorax, abdomen, legs, or wings- depending on the species (Tembhare, 2016). The table-6 shows some examples indicating the role of exocrine glands in pheromone communication in insects.

Table-6: Role of exocrine gland in insect communication

S.No	Exocrine gland	Location	Insect	Function	Reference
1.	Mandibular glands	At the base of mandibles	Hymenoptera *(Apis* spp.*)*	Queen substance	Mathur, 2010.
2.	Nassanoff gland	Abdomen	Hymenoptera *(Apis* spp.*)*	Aggreagtion pheromone	Mathur, 2010.
3.	Dufour's gland	At the base of the sting	Hymenoptera Ants (Only in females)	Trail Pheromone	Tembhare, 2016.
4.	Poison gland	At the base of the sting	Hymenoptera Ants (Only in females)	Defense	Tembhare, 2016.
5.	Pavan's gland	Below the sixth abdominal sternite	Hymenoptera *(Aneuretus simony)*	Trail pheromone	Billen *et al.*,2016.
6.	Stobbe's gland	In second abdominal segment	Lepidoptera Noctuid moths (male)	Sex pheromone	Tembhare, 2016.

7.	Atrial gland	Last abdominal tergite	Blattodea *(Periplanata americana)*	Sex pheromone	Tembhare, 2016.
8.	Pheromone gland	Hind tibiae	Hemiptera Aphids (female)	Sex pheromone	Tembhare, 2016.
9.	Scattered pheromone glands	Scattered all over body surface (head, thorax, abdomen)	Heteroptera *(Schistocerca gregaria)* Male locust	Pheromone secretion	Tembhare, 2016.
10.	*Coremata*	Posterior abdomen	Male arctiid moth	Aphrodisiac pheromone	Tembhare, 2016.

9.0 Differences between Insect and Mammalian Pheromones

The need of pheromone communication in insects and mammals are same. But changing physiology between mammals and insects causes the different pheromonal communication as mentioned in the table-7 (Wheeler, 1976; Liberles,2014).

Table-7: Differences between insect and mammalian pheromones

S.No	Properties	Insect Pheromones	Mammalian Pheromones
1.	Chemical composition	Use volatile organic compounds, such as hydrocarbons and derivatives	Use volatile organic compounds and also use complex mixture of peptides, proteins and other non-volatile compounds.
2.	Detection Mechanism	Receptors located on their antennae	Receptors located on VNO (Vomeronasal organ)
3.	Purpose	Mating, territory marking, aggregation and alarm signaling	Marking territory, establishing dominance and signaling reproductive readiness.
4.	Mechanism of release	Air borne	Air borne as well as exchanged through close

			physical contact
5.	Species specificity	Highly species specific	Species specific but some pheromones affects the other species (rarely)
6.	Complexity	Less complex in chemical composition	More complex in chemical composition

10.0 Sensory system in Insects to detect Pheromones

Insects have specialized sensory systems that allow them to detect and respond to pheromones. The main sensory systems involved in detecting pheromones in insects are:

1. Antennae

These are the essential organs to receive and detect the pheromones. The structure and arrangement of olfactory sensilla and olfactory sensory neurons (OSNs) on the antennae and palps of insects are very specialized and optimized to detect odorants, especially sex pheromones in the case of male antennae (Rizvi *et al.,* 2021).

2. Olfactory Receptors (OR)

It is present in the insect antennae. It detects airborne chemical compounds such as pheromones (Hansson *et al.,* 2011).

3. Pheromone Receptor Neurons

These are connected with the olfactory receptors. It links with the brain and transmits the sensory information from the receptors to the insect's brain. These receptor neurons are processing the pheromones, hence the name pheromone receptor neurons.

4. Sensory Structures on Legs or Mouthparts

Sensory structures are also present in the legs or mouthparts that also detect pheromones that are non-volatile through direct contact.

5. Pheromone Binding Proteins

These are otherwise known as odorant-binding proteins. These proteins are assisting to transport and deliver pheromone molecules to the olfactory receptors. These proteins play a role in concentrating pheromones at the receptors and enhancing their sensitivity.

11.0 Hormonal Control of Pheromones

The production and release of insect pheromones are often under hormonal control, primarily regulated by the insect's endocrine system. Hormones play a significant role in initiating, modulating, and coordinating the various stages of pheromone production and behavior. Here is an overview of how hormonal control works:

1. Juvenile Hormones (JH)

It regulates the development of sex pheromone-producing scent glands/ exocrine glands. For example, in female moths, juvenile hormones stimulate the development of the pheromone glands during the pupal stage.

2. Ecdysone

It is important for insect development and metamorphosis. It causes the maturation of insects. During maturation, the level of juvenile hormone decreases, and the level of ecdysone increases. In some insects, pheromone production is linked to specific developmental stages that are coordinated by the release of ecdysone (Morgan *et al.*, 1999). Lommel *et al.* (2022) reported that the knockdown of the ecdysone receptor in male desert locusts affects the relative weight of accessory glands and mating behavior.

3. Neurohormones

It also regulates the release of pheromones. For instance, in moths, pheromone production is triggered by the release of specific neuropeptides that stimulate the pheromone gland to synthesize and release pheromone compounds.

12.0 Applications of Insect Sex Pheromones

Studying insect communication specifically chemical communication and behavior will help to manage the particular insect pest. It will be eco-safe and species-specific and not harmful to beneficial insects. Further, these studies will also helps to monitor and survey insect populations, mass trapping, mating disruption, etc.

1. Monitoring

Pheromone-based traps are commonly used to monitor and study insect populations. These traps use synthetic pheromones to attract insects, allowing researchers and breeders to detect the presence, abundance, and distribution of specific insect species. This information is critical to make timely pest control decisions. Pheromone traps are now available for monitoring a wide variety of

pests, especially Lepidoptera, but also some Coleoptera and Diptera. To monitor the invasive red clover pests *Coleophora deauratella* Leinig and Zeller (Lepidoptera: Coleidae) in Canada, field experiments were conducted to optimize the trapping function of several pheromone baits (Mori *et al*., 2013).

2. Mass Trapping: Pheromone-based mass trapping uses synthetic pheromone-baited traps to attract and trap large numbers of insects. This technology can help reduce pest populations in certain areas, especially if the insects damage crops or cause economic losses. Mass trapping is a direct control strategy employing large numbers of pheromone traps to reduce population densities of target species and/or reduce pest damage (Jones *et al*., 1998; Trematerra *et al*., 2019). Mass capture was more efficient when both control methods had the same amount of pheromone sources compared to mating disruption (Byers *et al*., 2012). This is because disrupting mating only delays finding sex, whereas trapping delays it indefinitely.

3. Mating Disruption:

Mating disruption can be achieved by releasing synthetic sex pheromones into the environment, making it difficult for male insects to find females to mate with. This method reduces the number of successful matings, resulting in fewer surviving eggs and fewer pests over time. The most successful examples of mating disturbance pest control are the gypsy moth *L. dispar* (Lance *et al*., 2016); the codling moth *C. pomonella* (Witzgall *et al*., 2008); the grapevine moth *Lobesia botrana* (Gordon *et al*., 2005); and the Indian meal moth *P. interpunctella* (Hübner) (Trematerra *et al*., 2011).

4. Push-pull strategies

The push-pull strategy, in which both attractant and repellent stimulation are used to divert pests, is a sustainable alternative to traditional pesticides that are increasingly used. The strategy aims to reduce crop damage by altering the distribution of pests, using repellent stimuli to push pests away from the crop, while attracting stimuli to "pull" pests to areas other than the crop. Push-pull strategies have been developed primarily for agricultural systems to address the threat of insecticide resistance or reduce insecticide use (Rizvi *et al*., 2021).

Other applications are as follows:

5. Biological control

Pheromones can be used to increase the effectiveness of biological control methods. For example, the use of pheromone baits to attract natural enemies or parasitoid wasps to pest-infested areas can help increase their effectiveness in controlling pest populations.

6. Insects conservation:

Pheromones can also play a role in insect conservation plans. For example, in situations where locating and monitoring endangered insects is difficult, pheromones could be used to attract and detect them, helping to develop conservation strategies.

7. Research and Behavioral Studies: Pheromones are valuable tools for studying insect behavior, communication, and ecology. Researchers use pheromones to study mating behavior, territorial interactions, aggregation dynamics, and other aspects of insect social and reproductive life.

8. Development of novel control methods: Understanding the chemical composition and function of insect pheromones can facilitate the development of innovative control methods. This includes the development of more effective baits, new formulations, and the optimization of pheromone-based technologies.

9. Urban Pest Control: Pheromones are also used to control urban pests such as cockroaches and ants. Pest populations in and around buildings can be controlled in an environmentally friendly manner through the use of pheromone-based baits and traps.

Conclusion

Overall, insect pheromones provide valuable tools for sustainable pest management, scientific research, and various practical applications across different industries and fields. Application of pheromone in pest management is a rapidly evolving non-invasive and ecofriendly method and is going to be one of the most preferred modalities for pest control in the future. All the insects/ animals use chemical communication. Intensive research on identification, characterization, and associated behavioral study would give good solutions for managing insect pests. It will give a safe, species-specific, and eco-friendly pest management practices.

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"A Comprehensive Overview: Efficiency and Gear Selection in Fisheries"

Baig Laika Riyaz*, Saima Jan, Sumaira Rashid and Shafiya Mushtaq
Faculty of Fisheries, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Rangil-190006
*Corresponding Author: Baig Laika Riyaz (MFSc Scholar)
*Phone Number: 6006711135
*Email id: *laikabaig64@gmail.com*

Abstract

The main issue with fisheries now is that fleet capturing capacity is significantly higher than what is necessary to collect the resources that are currently accessible. There are numerous strategies for building a better future. Such a future would suggest flourishing fisheries. A fishing effort takes into account the available resources, wise financial decisions, a plentiful supply of jobs, safe and healthy working conditions, favorable market and trade circumstances, and last but not least, worthwhile and in-demand consumer goods. The combination of the following strategies can be used to achieve these goals. The first step is to limit fishing efforts and adjust them to the available resources, which frequently entails reducing current efforts and stabilizing them at a lower level. The second strategy is to improve the effectiveness of catching the desired species and size. This necessitates enhancing fishing gear's selectivity. Consequently, non-target species and sizes will have a greater impact on the bio-mass and recruitment of fish stocks.

Keywords: Selectivity, trawl net, gill net, FADs, artificial reefs

1. Introduction

The main objective of thefishermen is to obtain the highest possible return while putting the least amount of stress on fish populations and environment, which is the primary goal of any ethical fishing business. Quantifying the consequences of fishing on the environment and fish populations is significantly more challenging. The effectiveness of fishing has increased over time due to advancements in fishing gear and fishing methods, with little thought given to the effects on fish populations and fish habitat.

Making the gear selective could significantly lessen the impact of fishing on the population.The majority of fishing equipment is selective for fish within a specified length range, excluding very little and very large fish. Gear selectivity is the name given to this feature of fishing equipment. Thompson and Ben-Yami (1984) defined selectivity as the ability of any method or gear type to capture fish population, whether they are grouped by species, , size, age or behavior, while excluding others. For estimating the size composition of fish, gear selectivity is an important factor. The ultimate goal of research in size selection is to recommend appropriate mesh sizes to catch fish for the ethical exploitation of the stock (Sathianandan,2017).

When considering between-species selection, capture will mostly depend on how each species behaves towards the fishing equipment, whereas in the case of within-species selection, the retention of a fish will be determined by its unique traits (age, length, or girth). In this context, selection is frequently confused with length selection even though, in the context of meshes, selection mostly refers to girth/mesh-opening related processes. Selectivity is nothing more than selection expressed quantitatively.

2. Factors considered for designing or selecting fishing gear:

The target species' biological, behavioral, and distributional characteristics heavily influence the choice of fishing gear and its design. The design of fishing gear must take into account the existence of the greatest number of characteristics that are appropriate for specific fishing situation, resource, and any necessary trade-offs.Factors influencing the selection and construction of fishing gear are as follows:

2.1 Target species biology:

Biological traits, including body size and form, eating preferences, and swimming speed, have a significant impact on the design and selection of fishing equipment. The mesh size needed to enmesh and hold fish in gillnets and the mesh size needed to retain the target size groups of the species in trawls, seines, and traps without gilling are all influenced by body size and shape. They also affect the tensile strength requirements for the netting twine in gill nets and the hook size and lines in hook and line. Swimming speed is directly correlated with body size, making it an important factor to take into account when determining the fishing success of drifted gear. The target species feeding habits are particularly crucial when using active fishing techniques, such as troll line, to catch predatory fish, as opposed to passive fishing techniques like hook and line and traps, where the fish are drawn in by

the bait. The target species' swimming speed is crucial, especially when using active fishing techniques like trawling, seining, and trolling. By regulating the towing speed and reducing the longitudinal length of the trawl net, selective capture of the slow-moving crustaceans may be achieved, allowing the fast-swimming non-target finfish to escape.

2.2 Behavior of target species:

Diverse fish species exhibit diverse behaviors that might be utilized to choose the right equipment. As an illustration, various species' behavior may alter as they turn back into the trawl. Cod and flatfish are said to reverse direction in a horizontal plane at the bottom, whiting reverse direction at a level higher than this, and haddock rise and reverse direction at an even higher level. Using separator panels inside the trawl, it is possible to separate the various species as a result of this differential behavior. Designing a selective trawl could take advantage of differences in behavior and size between fish and crustaceans. Rigid grids are positioned at an angle and before the cod end in such designs. Fish and other non-target species are repelled by the grid and discharged via an escape chute when small prawns pass through it and into the cod end. The herding impact of the otter boards, wires, and sweeps, as well as the sand-mud cloud formed by the boards on finfish between the boards, are used in conventional trawling systems to increase the effective sweep area and increase catch rates. Squid jigging, light-assisted purse seining, and dip net operations all make use of some fish's propensity to gather near light. Fish Aggregating Devices (FAD) assisted purse seining by making effective use of the tuna's tendency to congregate around floating items.

2.3 Distribution of target species:

The vertical aperture of the trawl mouth, the vertical dimension in gill nets, and the catenary of the main line of the long line with branch lines and hooks all need to be in the vertical range of the layer with the highest concentration of fish to enhance catching efficiency. Vertical distribution of species can therefore be used to optimize the horizontal and vertical dimensions of the netting panels in gill nets, the mainline catenary in long lines, and the mouth design in trawls. Fish that are dispersed and scattered are more easily captured using passive fishing techniques like gill netting and long lining, but schooling fish are easier to catch using purse seining and focused mid-water trawling.

2.4 Fishing depth:

Deep-sea trawls, gillnets, and bottom vertical lines, buoyancy elements must be indestructibleso that it can withstand the high pressure at the depth. Compressible buoyancy elements, which are simple, inexpensive and light, can only be used in surface-operated gears like seines and surface gillnets because they absorb water and loosen under pressure.

2.5 Currents:

For efficient fishing, the right fishing equipment must be chosen based on the strength of the water currents. Prevalent strong currents in the fishing area may limit the type of fishing equipment available. In waters with high currents, you should utilize equipment that is less impacted by the current. Gillnets and Longlines are less susceptible to currents. Currents can easily disrupt drift nets.

2.6 Visibility:

Since light affects fish eyesight, light levels at the fishing depth may have an impact on fishing success. Fishing efficiency suffers when netting panels are visible in passive fishing gears like gill nets. While light-assisted jigging benefits from regulated lighting, hook and line operations are reported to be negatively impacted by visibility. During the catch process in trawls, huge pound nets, and trapping, visibility is also crucial for effective herding. The net color and tone contrast with the background, influenced by day time and seasonal fluctuations in water clarity or color, determine nets visibility of nets. Gillnets are built using fine twines that are selected to be largely invisible to fish, particularly at low light intensities. The color of nets can also affect visibility; according to studies, dark-colored nets can be used in clear water and light-colored nets in muddy water. The target fish swim into the net when it is invisible because they are unaware of its presence.

2.7 Sea bottom characteristics:

The usage of the majority of fishing gear near to shore is constrained by rough sea bottom conditions, with the exception of handlines, vertical longlines, bottom vertical long lines, and traps. Special rigging, such as a bobbin rig or rock hopper rig, changes in trawl design to reduce gear damage or loss, and the choice of proper otter boards are also necessary when trawling on rough bottom.

3. Trawl Net Selectivity:

The method of fishing, which involves pulling fish net through the water from the back of one or more boats, is known as Trawling. The net used in the process of trawling is termed a trawl net, whereas the used in fishing is termed a trawler. Bottom trawling and Mid-water trawling are major types of trawling. From the above two, bottom trawling causes damage to the sea floor by destroying the bottom environment. Trawl nets are used to catch different species, including squids, shrimps, cod, scallops and many more. Trawling also has a major impact on the catching of untargeted and undersized fishes, which serves as a by-catch. But, to overcome this, efforts can be made by reducing the mesh size of the nets and net structure. Different regulations are made in some countries to conserve sea beds and fish spawning grounds.

Trawlers come in a variety of sizes, and fishing can be done by a single trawler or by two trawlers (pair trawling) working together. Pair trawling involves the use of two trawlers with warps for the horizontal opening of the net. Single trawler fishing involves otter boards, also termed "Trawl doors," used for the horizontal opening of the net. Their trawl doors come in different sizes and shapes. These doors act as hydrodynamic wings and require a specific towing speed that generally ranges from 2.5 to 4.0 knots for better functioning. Trawl nets are tunnel-shaped and have a closed tail with one mouth opening wide. The vertical opening of the trawl nets is controlled by floating (placed on top of the net rope) and footrope (placed on the bottom of the net rope). Configuration of footrope varies on the unevenness of the seafloor. A number of variables, including towing speed, cable length, height of headline, water flow through the net, otter door spread and ground contact, affect the performance of trawling operation. Multi-species trawls can catch a variety of fish, depending on their size and form, in tropical and subtropical areas.Demersal fish species first encounter trawl doors before coming across the trawl net. Based on elements including light, turbidity, and their visual capabilities, fish may first hear the doors before finally catching a glimpse of them. A silt cloud that follows the doors causes disturbances in the water that may make swimming and breathing difficult for the fish and may be visible to them. Some fish escape by migrating away from the trawl, while others get caught in the trawl's path by the sediment cloud and possibly further guided between the doors (O'Neill and Mutch, 2017).

Two strategies are used to lessen this directing effect of fish from trawl:

a) Semi-pelagic doors, which do not come in contact with the seafloor
b) Doors designs that are mechanically raised from the seabed using skids

Trawling involves a lack of selectivity because a trawl net captures everything coming in its paths, including untargeted, undesirable and marketable species. All fishing techniques raise the risk of catching undesirable species. The concern is shared by both fishermen and ecologists. It also catches fish of both legal and illegal sizes. Occasionally, while fishing, some by-catch species get injuries, and some may die. Common by-catch species include sharks, dolphins and sea turtles. Trawling also results in large volumes of by-catch. Mesh size in the cod end of the trawl net determines size selectivity of species. Fishermen deal with difficulties because some fish that are legal size escape through mesh sizes that allow undersized fish to do so. Many methods are used to solve this problem, like tying ropes to stop the mesh from opening completely. The issue is that the mesh gets distorted when a trawl net has diamond-shaped (rhombuses) mesh rather than square-shaped mesh (Boopendranath and Pravin, 2005). By-catch reduction grids and square mesh panels are two examples of devices that let some species evade capture while keeping others in. According to some studies, shrimp trawling results in the greatest amounts of by-catch (FAO, 2007).

Selectivity of trawls refers to the ability to catch various sizes of fish species. For measuring selectivity, several methods are employed, which are mentioned below (Holt,1963):

2.8 Covered cod end:(Sistiaga*et al.*, 2009)

Procedure: In the trawl net, a small mesh cover is attached over the cod end. The small fish population that escapes from the cod end are caught and retained by the fine mesh cover, which gives information about the entire population. The selectivity of experimental cod ends can be calculated using different cod ends with variable mesh sizes and shapes.

Advantage: This method helps to evaluate size and distribution of the total fish population that encounters the gear in a specific fishing zone.

2.9 Twin Trawl:

Procedure: In this, two trawl nets are simultaneously dragged by one trawler. By studying length frequency distribution of the cod captures from both the experimental and small mesh cod-ends, selectivity is determined. With this technique, selectivity estimation bias will be reduced.

Advantage: One trawl cod end with a small mesh is used to estimate the total population, whereas the other serves as an experimental gear.

2.10 Trouser Trawl:

Procedure: In this method, a standard trawl is divided into two sections by using a vertical panel, each with its own cod end.

Advantage: Enable comparison of length-frequency distribution and estimation of cod end selectivity.

2.11 Alternate Hauls:

Procedure: In this method, identical hauls are alternately made by using an experimental and control trawl. To ensure the reliability and validity of the data, each pair of hauls should be identical, including the number of hauls made.

Advantages: Selectivity data is collected in a controlled and consistent manner.

2.12 Parallel Hauls:

Procedure: In this method, two trawlers simultaneously perform fishing operations in the same area. The control gear is towed by one trawler, while the experimental gear is towed by the other. Thus, with the help of this, researchers can assess the selectivity of experimental gear by contrasting the catches from both the trawlers.

Advantage: It is used to assess gear selectivity in the real fishing environment.

All these techniques are crucial for studying the operation of trawl gear and formulating strategies to reduce bycatch, safeguard young fish, and advance sustainable fishing methods (Madhu, 2018).

4. Bell-shaped selection curve:

A shaped curve is significant for the study of the selectivity of various fixed gears. The selection range of the gear is provided by the breath of the selection curve, and the highest point in the curve corresponds to the ideal size of fish caught by the gear. A monotonous selection curve cannot adequately simulate a bell-shaped selection curve. A more flexible empirical size selection model can manage a bell-shaped curvature (Lovgren *et al*., 2016).

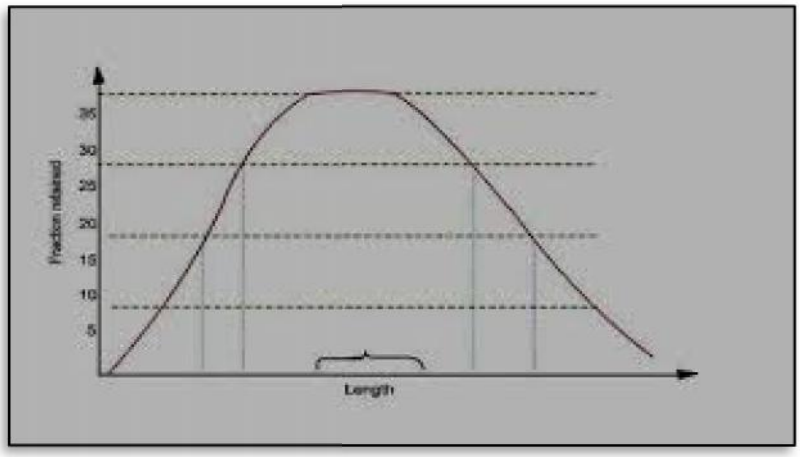

Figure-1: Bell-shaped selection curve (Source: Internet)

Two selection devices includesa grid system and a standard cod end theywere mounted consecutively in order to provide a bell-shaped selectivity for a target species in trawl fisheries. All fish should ideally come into touch with the grid while swimming normally, and the grid should then sort the fish by size. Regardless of their size, some fish entering the gear may escape through the outlet because not all fish entering the gear will definitely contact the grid (Sistiaga*et al*., 2010).

5. Sigmoid shaped selection curve:

Selectivity curves, which quantify the likelihood that a specific length class of a given fish species would be captured, given that it is available to the gear, are used to characterize the size selection of fishing gear. Different gear types and gear configurations have different selectivity curves (Smith and Walsh,1996). Larger fish cannot enter the fixed gear, but smaller fish can escape because of their relative size. Mobile gears, in contrast, allow all of the smaller fish to escape via meshes, making larger fish more vulnerable to selection. The proportion of fish kept increases along with fish size. The selectivity curve is "S" shaped as a result of the selection process. The 50% selection size is the size at which 50% of the fish are kept and 50% escape. The efficiency of the selection access is indicated by the curve's steepness. Poor selection access is represented by a shallow curve that gradually shifts between and, whereas ideal selection is represented by a curve that abruptly changes from 0% to 100% selection (a vertical line). The vertical line selection that

results from the limited selection range is said to resemble a "knife edge" pattern (Huse *et al*., 2000).

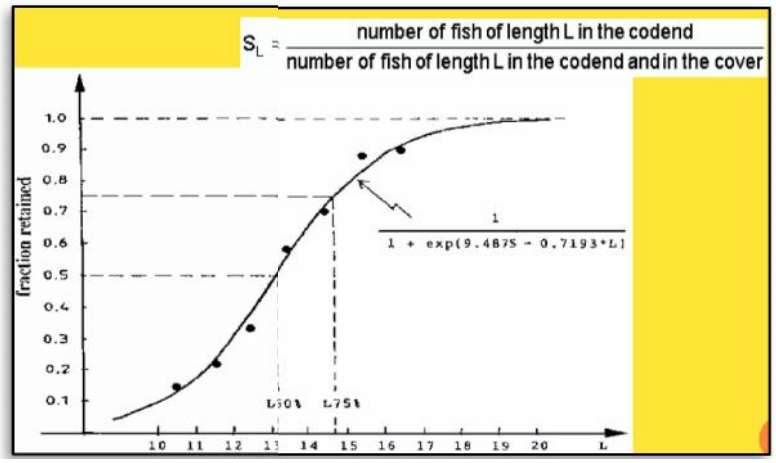

Figure-2: Sigmoid shaped selection curve (Source: Internet)

6. Gill net selectivity:

Gillnets are effective at catching widely dispersed species, such high seas salmon, and cost-effective in small lakes or underdeveloped regions where they may be caught from small boats with a low investment in labor and equipment. Although gillnets are a popular research method for sampling fish populations, they are quite picky. As a general rule of thumb, few fish are caught whose length varies from the ideal by more than 20% (Baranov, 1948). Therefore, the size distribution of a capture may not be a good representation of the population being sampled. In gillnets, fish get caught by gilling or wedging and tangling (Hamley,1975). Selectivity can impact any estimation that include random sampling, such as length-weight regressions, sex ratios, estimates of population size determined by capture-recapture methods, and growth and mortality calculations (Ricker, 1969). In order to manage a commercial gillnet fishery to produce maximum yield and selectivity, knowledge is important.

Awareness of gillnet selectivity includes the following: (Baranov, 1914)

a) Fish enters a mesh above the gill covers cannot entirely pass through, it is trapped.

b) Selectivity curves are comparable (same size and shape) with different mesh sizes.

The majority of subsequent studies continued to be based on these generalizations. Since estimating the shapes of selectivity curves is challenging, attention until the 1950s was focused on figuring out the best mesh sizes for certain fisheries. Soviet researchers estimated modes of selectivity curves from catches using two more mesh sizes under the congruity assumption (Andreev, 1955), while Western and Japanese scholars, the majority of whom were unfamiliar with Baranov's study, deduced selection ranges from earth measurements or the size range of fish collected (Konda, 1962). Other than mesh size, the elements that most affect the effectiveness of the net may also affect selectivity.

6.1 String color:

The color of the string has an impact on the performance of gillnet fish. Water clarity, lighting, and the wavelength of light reflected by the twine all affect how visible nets are in the water. Steinberg (1964) demonstrated that catch efficiency is affected by water clarity, which varies depending on the species individual visual acuity. Hence, gillnets are mostly used from dusk to dawn. Baranov (1948) observed that the color of the dorsal region of the fish is the most efficient color of a net.

6.2 Lunar phases:

Collins (1979) found that the lunar phases are related to fish catch. The relationship is explained by increased illumination around moonlight, which increases fish rejection of the nets by making them more visible (Blaxter and Parrish, 1965). Monofilament twine has better fishing efficiency over multifilament nylon was mainly due to its low visibility. The procedure of "hanging," which refers to the connecting of the webbing to mainlines or cords enclosing the net, has an impact on gillnet ability to catch fish. A net that is "hung" by half has a final length that is 0.5 times the stretched netting's length, while a net that is "hung" by a third has a final length that is 0.667 times the stretched netting's length (Molin, 1953).

6.3 Duration of the set:

The duration of the set has an impact on the efficiency of gillnets fish. Kennedy (1951) discovered that high catch rates gradually decreased the effectiveness of gillnets as a fishing tool. The saturation level of the catch was also discussed by Pycha (1962) both pointed out the need to determine an effective fishing effort by comparing the duration of the set to saturation restrictions. The presence of deteriorating fish in gillnets that are left out for a longer amount of time may lower the catch efficiency.

6.4 Non-catch mortality by gillnets:

- Ricker (1976) conducted in-depth reviews of non-catch mortality in gillnets for fisheries along the Pacific coast. Although some observations on gillnet escapees (fallouts) have been made, they are not much concrete proof of the mortality (French and Dunn, 1973).
- According to Petrova (1964), more net-marked salmon than unmarked salmon are killed in fresh water. Despite the fact that many estimates cited by Ricker (1976) suggest that declines from non-catch mortality surpass 50% of the catches, these losses appear to be limited to salmonids. For inshore coastal areas, non-catch mortality in gillnets was relatively low (1-2%),
- Jewel (1970) reports. They suggest that if gillnet fishing is limited to protected coastal seas, CPUE may increase.

6.5 Use and Significance of Gillnet Selectivity:

- By selecting the appropriate mesh size for the available population, improved management of a commercial fish stock is possible with knowledge of the selectivity of any gear, including gillnets. Additionally, it allows for a commercial catch information-based independent estimation of the population structure, which is not possible with unadjusted gillnet catch data.
- Unselective gear or adjusting the catch to account for selection is required for unbiased sampling of a population.The catch duration distributions vary more as the variation between mesh sizes grows. The summed selectivity must be divided by the overall catch frequencies in order to remove or minimize this variability, with the resulting distribution being normalized to 1.0. Through this process, gillnet capture data can be used for studies on ageing and as a source for catch curves for mortality estimations.
- Experimental fishing with a fleet of gillnets of different mesh sizes will provide the data for estimating optimum fishery selectivity to remove the desired number and size of fish.
- Clay (1979) revealed a significant amount of variance in trawl codend selectivity.

7. Selectivity of fish attraction methods:

The attractant, fishing equipment, location and various methodall work together to produce selectivity. The major strategies for attracting fish are using light, bait, floating rafts, and artificial reefs.

7.1 Light attraction:

In the tropics and subtropics, lights are employed to draw fish into purse seines, ring nets, and lift nets. Due to fish reactions that can be unfavorable or unpredictable, light attraction is not frequently used in temperate waters. In many instances, juvenile fish are drawn to them, while mature fish are either repulsed or unaffected. The majority of tropical small pelagic fish exhibit positive phototaxis.Sardine, anchovies, saury, small mackerel, and bait fish are some of them. Squids are drawn to light as well, although they like to hang out on the edges of the illumination. The abundance of small fish and their movement or heightened activity in the presence of light attracts larger fish. Therefore, captures made with light attraction and seines or lift nets typically consist of a variety of fish species. Squid, which are caught using specialized jigs that won't capture other species, and surface-swimming saury, which are caught with stick-held dip nets in waters north of Japan, are exceptions to this rule. The composition may vary from region to region. Using the same nets and lights in shallower or deeper waters may result in catching different fish. Artificial illumination has been widely employed in fisheries and the aquaculture industry for attracting and baiting fish species. Achari *et al*. (1998) employed kerosene lamps and natural gas lamps to entice and capture fish inIndia and discovered that the use of underwater lights was substantially more effective than lights put above the surface. New light-emitting diode (LED) lights have also gained popularity in recent years as a fish attractor. Fish will be drawn to and directed by bubble curtain configurations in different ways.

7.2 Bait attraction:

The use of bait to draw fish may be as old as fishing itself, and it is still commonplace today, especially in traps and pots used to catch lobster, crabs, prawns, and rockfish. After a downturn for a few years, long lining for cod, halibut, ling, or dogfish is once again being used in northern waters. In the Pacific and Indian Oceans, tuna long line is still a common technique. Off the eastern coast of the USA, swordfish long lines are utilized. In several regions of the world, small-scale fisherman employs bottom-set long lines. The majority of these techniques are largely species-specific. Crabs in lobster pots

skate on cod lines, and sharks on tuna lines are still occasionally encountered, although they remain the exception rather than the rule. It is possible to think of baited lines and traps as selective in both their attraction and their capture. Of course, the fact that they are placed in places and at depths where the fish sought are known to exist plays a part in this. Skipjack, bonito, and other schools of tuna are attracted to live bait fish, which are then caught using a pole and line. This is a fairly selective strategy since the fisherman can see what they are capturing and because the schools are initially identified visually. Some of the live bait is captured by the bait itself, which is a powdered meal made of cereal, fish, or other protein. Chumming is the common name for the usage of this type of bait, and it is typically selective in drawing only the small bait fishes required by the pole and line vessels (Thompson and Ben-Yami, 1984).

7.3 FADs and artificial reefs:

FADs, or fish aggregation devices, are used in the tropics to draw pelagic fish. Boats fishing with pole and line and tuna purse seines frequent the deep waters off the Indo-Pacific islands. Smaller lift-net boats and purse seiners fish in the vicinity of FADs anchored in shallower waters close to shore, where sardines and small mackerels may be present. The FAD is a floating raft on which cords made of palm leaves, discarded ropes, netting, or tires are fastened. It is unclear why fish find these devices so appealing. It might be done in part for protection or shelter, in part for navigation, and in part to eat the smaller fish or algae that gather there.

The procedure of attracting fish to artificial reefs is undoubtedly crucial. The development of adult fish assemblages on artificial reefs typically occurs shortly after installation (Folpp*et al*. 2011), which is consistent with most definitions of "attraction." Simple "thigmotaxis" (Brickhill *et al.* 2005) or "instinctive orientation" (Pickering and Whitmarsh 1997) may be the source of attraction, but "behavioural preferences" (Bohnsack 1989), which are frequently cited as a source of attraction, might not be separate from fish production. Fish output may increase through higher development or survival if a newly placed artificial reef provides the attracted fish with some increased value, such as a better refuge. However, estimating risk requires differentiating between redistribution with and without benefit to production, which is notoriously difficult. However, an alternative is to model attraction and subsequent fishing catch. Numerous fish species have been taught to link sound signals with either fear Hawkins and Sand (1977) or food (Abbott, 1972). Before being released into a tiny bay in Japan, juvenile red sea bream (Pagurus

major) was trained to correlate sound signals with food, demonstrating for the first time the value of acoustic training with fish. Social learning was demonstrated three months later when a significant portion of the conditioned fish responded to the signals, and some wild red sea bream were drawn in as well (Fujiya *et al*., 1980).

8. Conclusion

A key element for sustainable resource management is the choice of fishing gear. A thorough examination of the numerous types of fishing gear, their designs, and operational systems reveals that gear selectivity is a broad concept. Selective gears that lessen bycatch and encourage the escape of juvenile or non-commercial species are beneficial for both the preservation of marine biodiversity and the long-term health of fish populations. The issue of efficient gear selectivity does not have a single solution. It needs ongoing innovation, creativity, and technical change. The delicate balance of marine ecosystems is threatened by overfishing, habitat destruction, and climate change hence, to overcome this gear selectivity is emerging as a critical tool for responsible fisheries management. Understanding and improving gear selection will remain important as advance in our efforts to promote a peaceful coexistence between human livelihoods and the protection of our waters for future generations.

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Oral Mesenchymal Stem Cells

K. Tamil Ilakkiya
CRI, Priyadarshini Dental College and Hospital
Phone 6383116564
Email: *tamil8kalai@gmail.com*

Dr. Pavani Muddepalli
Associate Professor,
Department of Oral Medicine and Radiology,
Priyadarshini Dental College and hospital
E-mail: *Pavani.m433@gmail.com*

Introduction

The development of biotechnologies in recent decades has the potential to revolutionize biomedicine through the use of cutting-edge protocols.[1] These lofty objectives, which were enthusiastically set in late 1990, appear to have finally been realized in the dentistry area, which may be close to achieving significant outcomes.

For the study of tissue regeneration and the application of regenerative medicine, stem cell biology has grown in importance. Since the identification and characterization of multipotent mesenchymal stem cells (MSCs) from bone marrow (BM), MSC-like populations from different tissues have been assessed in accordance with the 'gold standard' criteria established. [2,3] These MSCs have the capacity to generate at least three distinct cell lineages, including osteogenic, chondrogenic, and adipogenic. BMMSCs may potentially give rise to other lineages, such as myogenic, neurogenic, and tenogenic. In recent decades, a variety of stem cells have been found in every organ and tissue of the body as a result of the hunt for MSC-like cells in particular tissues. [4,5]

These MSCs have the capacity to generate at least three distinct cell lineages, including osteogenic, chondrogenic, and adipogenic. BMMSCs may potentially give rise to other lineages, such as myogenic, neurogenic, and tenogenic.[6] In recent decades, a variety of stem cells have been found in every organ and tissue of the body as a result of the hunt for MSC-like cells in particular tissues. [7]

According to Moraleda et al [8] (2006), stem cells are clonogenic, self-renewing progenitor cells that have the capacity to produce one or more specific cell types. ES cells, or embryonic stem cells, and AS cells, or postnatal or adult stem cells, are the two basic categories of stem cells. The inner cell mass of a blastocyst, an early, pre-implantation stage embryo, is where ES cells, or embryonic stem cells, are produced. The self-renewing multipotent stem cells known as Adult Stem cells are found in the majority of differentiated tissues and organs. It is believed that AS cells move to the site of injury and specialize into particular cell types to aid in tissue repair. [9]

Types of oral mesenchymal stem cells:

Almost all tissues include adult stem cells, which are undifferentiated cells. [10] and have the ability to renew themselves as well as develop into a variety of histo-types.[11] These cells serve as the foundation for any tissue engineering technique. The bone marrow (BM), which contains hematopoietic stem cells (HSC) [12,13] and mesenchymal stem cells [14] is where stem cells were initially discovered. MSCs are abundant in the oral cavity, where they are found in specialized, well-recognized tissues. [15]

"Postnatal dental pulp stem cells" (DPSCs), the first variety of dental stem cells, were discovered in the pulp tissue of a third molar in a human.[16] Later, additional types of dental MSCs were described in accordance with the various isolation sites, including buccal fat pad (BFPSCs), periodontal ligament (PDLSCs) [17], apical papilla of developing teeth (APSCs), dental follicle (DFSCs), gingiva (GFSCs), and pulp tissue of exfoliated deciduous teeth (SHED).[18]

Dental pulp stem cells:

The DPSCs are a population of clonogenic cells with a strong proliferation capacity found in the dental pulp of adult teeth.[2] After separating the crown from the roots, these cells were successfully extracted through the enzymatic digestion of pulp tissue. Studies conducted in vitro and in vivo have demonstrated the plasticity of DPSCs. In vitro, DPSCs can differentiate into chondrocytes, osteoblasts, odontoblast-like cells, adipocytes, neural cells, cardiomyocytes, and myocytes.[19] In vitro, under osteo-inductive conditions, DPSCs can produce mineralized nodules with a dentine-like structure, and in vivo, they can produce reparative dentine-like tissue on the surface of human dentine. In vivo, DPSCs implanted with the carrier hydroxyapatite/tricalcium phosphate (HA/TCP) develop a dentine-like structure surrounded by pulp-like interstitial tissue and lined with human odontoblast-like cells.[20]

Stem cells from apical papilla:

The apical papilla of human immature permanent teeth has a stem cell that may be a novel kind.[21] The apical papilla is different from the dental pulp in that it serves as a precursor tissue for the radicular pulp. SCAPs are created from a growing tissue that may represent a population of early stem/progenitor cells and are produced by explant cultures or the enzymatic digestion of apical pulp tissue.[22] The soft tissue near the tips of growing permanent teeth is called the apical papilla. This tissue represents the radicular pulp's precursor tissue and is rich in stem cells with a strong proliferation potential. Soft tissue that is loosely linked to the apices of developing permanent teeth, such as the third molar, makes it simple to obtain SCAPs. SCAPs have been examined for their capacity to regenerate since the dental papilla is the tissue that creates the dentin-pulp complex.[23]

Additionally capable of osteo/dentinogenic, neurogenic, and adipogenic differentiation are stem cells from the apical papilla. Although these markers are expressed at lower levels in SCAPs than in DPSCs, SCAPs exhibit an expression pattern of osteo/dentinogenic markers and growth factor receptors that is comparable to that seen in DPSCs. Despite these results, it is still unknown how well SCAPs may differentiate into myogenic and chondrogenic tissues.[24]

Periodontal ligament stem cells:

PDLSCs are a subset of stem cells found in human PDL that exhibit MSC surface markers, self-renewal capacity, multipotency, and the ability to develop into adipocytes, cement oblasts/osteoblasts, and collagen-forming cells. [25,26] Thus, PDLSCs are in charge of masticatory function, tooth-bone attachment, and regeneration and maintenance of periodontal tissue homeostasis. PDLSCs are the most researched and regarded as the best source for periodontal regeneration since they can release mineralized structures and are readily available.

It is possible for periodontal ligament stem cells to develop into cells that resemble cementoblasts and collagen-producing cells. Compared to what has been seen with DPSCs and SHEDs, the formation of calcified nodules is less apparent. PDLSCs can also undergo in vitro differentiation into adipogenic, osteogenic, and chondrogenic cells. [27,28]

PDLSCs may produce cementum/PDL-like structures in vivo when transported by hydroxyapatite/tricalcium phosphate (HA/TCP).[29] Recently, there has been a lot of interest in the secretome of PDLSCs as well. The ability

of PDLSC-conditioned medium (CM) to stimulate new PDL attachment and bone defect healing in rats with periodontal defects has been studied. More recently, a mixture of concentrated growth factor and PDLSCs-CM was beneficial in stimulating PDLSC cell proliferation, demonstrating this product's usefulness for upcoming applications in periodontal tissue regeneration, according to Nagatai et al. [30]

Stem cells from exfoliated deciduous teeth (SHED)

SHED is derived from tooth pulp, just like DPSCs. However, in comparison to DPSCs, SHED expresses higher quantities of genes related to stemness (OCT4, SOX2, NANOG, and REX-1), maintaining higher flexibility across passaging in vitro because of the developmental differences between deciduous and permanent teeth. [31] SHED is highly proliferative and capable of differentiating into a variety of cell types, such as neural cells, osteoblasts, chondrocytes, and adipocytes. Human deciduous teeth may be used to obtain stem cells, according to a 2003 study by Miura et al [32] These multipotent cells with immunosuppressive qualities are produced from dental pulp explants or by digesting dental pulp tissue as DPSCs.

Even though they may not be as effective at repairing damage as odontoblasts formed from DPSCs, SHED can differentiate into them;[33] In fact, they produce dentin-like or pulp-like tissue but not the dentin-pulp complex. Because SHED only forms the dentin-pulp complex when mixed with collagen I and injected into fully developed human root canals, this method may be used to speed up the creation of roots in necrotic, immature permanent teeth.[34]

Dental follicle stem cell:

Alveolar bone and the root-bone contact are formed by DFSCs, which live in the connective tissue loosely surrounding the growing tissue. The removal of teeth is related to their retrieval.[35] The proliferative potential and osteogenic properties of DFSCs are higher than those of the other dental MSCs. [36,37] Compared to PDLSCs, DFSCs are more immature and express more DSPP. They can regenerate dentin and may also be capable of periodontal differentiation and root regeneration, demonstrating a clear odontogenic potential.[37] In vitro, DFSCs can create PDL-like structures.

Gingival mesenchymal stem cells:

Mitrano et al [38] identified and characterized GMSCs; they exhibit multilineage differentiation abilities, express MSCs markers, and increase adherence, which are the minimum requirements for MSCs. GMSCs have high accessibility compared to other dental MSCs and do not require tooth extraction for harvesting. GMSCs are easily accessible from healthy or inflamed gingiva and are frequently discovered in scraps of tooth tissue.[39] They generate an anti-inflammatory macrophage polarization and inhibit osteoclasts, lowering periodontal bone resorption in a mouse model. GMSCs demonstrated immunomodulatory activity similar to that of the other dental MSCs.[40]

Application of mesenchymal stem cells:

The ability of adult MSC populations to easily differentiate into osteogenic and chondrogenic cells has garnered interest since they were first discovered in bone marrow because of their potential utility in bone and cartilage repair. Similar to this, dental MSCs, and more especially DPSCs, have the clinical ability to restore the pulp after endodontic therapy (also known as root canals) and to biologically promote tooth repair through the production of reparative dentine.

Restoration of dental pulp

The most evident application of cultivated DPSCs is the restoration of tooth pulp after root canal therapy. The present method of treating infected root pulp entails physically removing it and then sterilizing and filling the root canals with cement. Such treatments do not revive the vitality of the tooth or replace damaged dental pulp tissue while being efficient in battling illness. The use of DPSCs to restore healthy pulp tissue is a straightforward biological remedy with a high potential for effectiveness. In vitro expansion of DPSCs is simple, and both ex vivo and in vivo reconstitution of pulp-like tissue have been demonstrated. [2,18]

Misako Nakashima is now examining the viability of employing dental pulp stem cells to restore infected pulp tissue in a small-scale Phase I clinical experiment in Japan. In the trial, autologous "mobilized" DPSCs are used to treat patients with irreversible pulpitis, a disorder marked by persistent sensitivity to particular stimuli, on their teeth. There were no negative side effects after 25 weeks, and the treated teeth recovered their pulp. [41]

Craniofacial skeletal repair:

Adult MSCs from DPSCs may still have some characteristics of the neural crest cells from which they were derived. Other adult mesenchyme-derived stem cells keep a memory of where they came from, which may affect how they differentiate. This is important for applications involving bone tissue creation.[42] While neural crest cells that form the more caudal skeletal structures and mesodermal-derived skeletal cells express Hox genes during development, cranial neural crest cells that form the face and jaw skeleton do not, which suggests that a memory of this expression is retained into adulthood.[43]

The majority of the bones in the skull and face are made by osteoblasts from the neural crest, which have been said to differ from osteoblasts from the mesoderm. To make membrane bone, it may not be best to use skeletal stem cells, as an example in craniofacial bone tissue engineering. Similar to this, the use of mesenchymal cells derived from the neural crest may be advantageous in the creation of cell-based organ engineering systems to produce tooth primordia that can develop into teeth after transplantation. An accessible source of these cells is the tooth pulp. The degree to which tooth pulp-derived MSCs can be forced to adopt a non-neural crest-like state, either by changing their local milieu or by direct cell reprogramming, may determine the extent to which they can be employed to create skeletal tissue outside of the craniofacial environment. The commercial banking of these cells, either from naturally exfoliated teeth (children's deciduous teeth) or from extracted teeth, is a result of these and other hypothesized therapeutic applications of DPSCs. To provide a banked source of autologous cells collected with no assistance or damage, pulp cells are cultivated and kept for later use.

Periodontium:

The periodontal ligament, a complex connective tissue, connects the jaw's alveolar bone and tooth roots. Its function is to cushion the forces produced during chewing and to seal the teeth, guarding the roots. In the industrialized world, periodontal disease is a significant factor in tooth loss, and the complexity of the tissue makes it challenging to restore sick or injured periodontal tissue. PDLSCs, which are increasingly being researched concerning the treatment of periodontal disease, can be produced through in vitro cultivation of cells obtained from periodontal tissue. [44,45]

This cell population may consist of cementoblast precursors, the cells that create the mineralized cementum connecting the periodontal ligament to the tooth, as well as connective tissue-derived cells from the ligament. The most likely candidates for producing cementoblasts and osteoblasts as well as the soft tissue cells of the ligament during repair are PDLSCs. Although lineage tracing has yet to provide proof, it might also play a role in healthy tissue turnover. In general, there aren't many in vivo characterization studies of PDLSCs, leaving fundamental concerns about their genesis, distribution, heterogeneity, and capacity for in vivo differentiation unsolved.

Mineral initiation:

Unfortunately, the incorrect belief that all cells with these features are 'equivalent' in their capacity to form osteoblasts and create bone has been caused by the basic criteria outlined for classifying cells with MSCs characteristics in vitro. Cell growth in an environment that induces osteogenesis is tracked by tests for the expression of osteogenic genes (such as alkaline phosphatase and Runx2) and/or mineral deposition using Alizarin Red staining as the gold standard. Ex vivo techniques are rarely employed to determine mineral formation, but when they are, they clearly show significant differences in mineral creation from MSCs of various sources.[2]

Autoimmune and inflammatory disease:

Autologous/allogenic MSCs are effective in treating autoimmune and inflammatory illnesses in humans because of their immunomodulatory action and minimal immunogenicity. Clinical trials using intravenous or local injections of MSCs in patients with systemic lupus erythematosus [46,47], rheumatoid arthritis [48], GVHD [49], and osteoarthritis [50] have shown their clinical efficacy and safety. The patients' disease activity scores decreased, the T cell imbalance was rebuilt, and their functional abilities improved. However, it was suggested that MSC transplantation would not be successful in refractory patients in the long run despite the encouraging results in clinical trials.

Conclusion

Teeth can be used to obtain readily available multipotent stem cells that can develop into several cell types. The development of methods for use in regenerative endodontics and degenerative disorders may eventually be aided by this novel source of stem cells. Future research will likely concentrate on stem cell subpopulations produced from teeth that are distinguished by various

surface markers, their unique characteristics, and their usage in clinical applications.

Oral MSCs have been suggested as the best candidates for MSC-based tissue regeneration because of their exceptional tissue reparative/regenerative ability and unique therapeutic advantages of easy access in large quantities. Additionally, the state of our knowledge indicates a striking biological interaction between oral MSCs and their inflammatory environment. Therefore, a greater comprehension of the underlying processes behind the immunomodulatory effects of oral MSCs may help to advance MSC therapy towards practical application.

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Automation in Medical Microbiology

Ms. Jasmine Vinshia, MSc, (PhD)
Lecturer of Microbiology
Rajas Dental College & Hospital
Contact: +91-8778255983
E-mail: *jasminevinshia@rajasdentalcollege.edu.in*

Introduction

Automation is the application of technology, programs, robotics or processes to achieve outcomes with minimal human input in a manufacturing or work process.[1]

Clinical chemistry and clinical haematology departments of diagnostic laboratories have gradually automated in recent years, however clinical microbiology laboratories have mainly been exempt from thisdevelopment.[2]

Despite the fact that some larger microbiology laboratories use automated systems for blood culture, for efficient microbial identification and for performing hassle-free antimicrobial susceptibility testing, a majority of microbiology specimen processing and culture workup are still carried out manually.With the exception of a few labs in Western Europe, Australia, and the Middle Eastern countries, the majority of microbiological labs practice least automation in their sample-processing units and as Total laboratory automation (TLA) concept is still not implemented in such laboratories. [2]

Microbiology laboratories are experiencing a rapid transformation, with several changes presenting challenges for researchers. This includes an increased complexity and an increased cost effect in the laboratory procedures in routine basis. These pressures and challenges facilitated the need for emergence of consolidation and centralized facilities in microbiology labs for reducing costs, improving turnaround time, and improving testing efficiency.[11,12]

Conventional Identification Methods (In-Use)

Sample collection: Appropriate samples are collected manually depending upon the type of infection. The samples are inspected manually by a trained professional and selected for further processing.

Media preparation: Culture media to be prepared is decided depending upon the type of sample collected. Care must be taken while weighing out the exact quantity of agar and other essentials to be added to the mixture.

Sample inoculation: Care must be taken to avoid contamination. Trained professionals inoculate the sample onto appropriate agars and incubate under suitable temperatures for further growth and identification of bacteria.

Phenotypic identification: The metabolic differences between different microbial species are identified using previous knowledge of cultural characteristic and outcomes of Gram staining etc.

Biochemical analysis: As the different tests are carried out, the results obtained narrow the possible options until an identification is obtained. For this, variety of biochemical reactions are preformed depending upon the bacteria isolated using culture media.

Limitations Of Conventional Identification Methods:

1. Time consuming: It takes around 24-48hrs for a confirmed identification of a microbial culture. This simultaneously becomes hectic when done in routine basis when reporting numerous number of clinical samples.

2. Increased workload: The diminished amount of trained professional in the laboratory sector invariably pressurizes the existent technical staff working in a shift basis. Thus an increased workload is infused on them which may directly affect the work outcome.

3. Professionalism: Only trained technical staff can carry out the tasks related to workflow and logical thinking and prompt decision making ability is highly required for a smoother run of the laboratory.

Impediments To Automation[2]

These include the ideas that:

1. Microbiology is too complex to automate,

Microbiology specimens are considered to be much more complex due to

a. Varieties of specimens to be collected

b. Specific considerations while sample collection for each sample
c. Complex transport criteria
d. Issues related to appropriate media selection for suitable sample processing

2. Humans are irreplaceable,

The perception that machines cannot exercise the critical decision-making skills and its inability to think and perform tasks demotivates machine interference in laboratories.

3. Automation is too expensive,

Historically automation has been considered too expensive since relative specimen and test volumes for microbiology are much smaller, making automation seemingly less attractive.

Requirements For Automation(Winds Of Change)

Several driving forces that are changing attitudes about automation in microbiology laboratories have emerged. These relate to overall changes in the laboratory industry, growing shortages of trained personnel, declining reimbursement, a growing demand for improved quality, and emergence of newer technological innovations. To cope up with these a microbiology lab should be flexible in design, embrace the human element, and adapt to the challenges of specimen diversity.

Some of the Automated Innovations include:

A. Staining

i. Automated Gram stainer

Bacterial staining plays a vital role in every microbiology laboratory and is one of the basic steps in disease diagnosis. Automation makes Gram staining easy and safe while ensuring accurate and standardized results in minutes**.**

The most recent automated machines include:

1. ColorAX2[3]

ColorAX2 uses 10 individual staining chambers for single slides. These chambers are filled with the staining reagent and automatically drained after staining. The compact, lightweight and autonomous system features 10 independent staining chambers and allows easy and clean procedures. The unique technology of ColorAX2 prevents cross-contamination and significantly reduces the consumption of reagents and waste. Besides it helps you reduce your operation costs.

2. Previ® Color Gram[4]

PreviColor Gram sprays the reagents on the slides in a centrifuge system with a capacity of 12 or 30 slides.

Advantages of Previ over ColorAX2

- Standardized staining – innovative spray nozzles always dispense the same reagent volume
- No cross contamination – each slide separated & fresh staining reagent used each time
- Improved microorganism differentiation compared to manual and bath staining results

B. Automated Culture Media Preparation and Dispenser System

In the past few years, biological science technology has advanced to unprecedented heights. Since media preparation is time-consuming and requires extra efforts, it forms the most challenging field of microbiology where automation mostly desired. An automated culture media preparation and dispenser unit must include prepare, sterilize, and dispense the prepared media in Petri plates or test tubes. The steps include:

- Weighing the powder form of the desired media
- Measuring the appropriate amount of distilled water
- Dissolving the powder in distilled water completely by application of heat
- Sterilizing the mixture in an autoclave
- Addition of supplement (if any) like blood in Blood Agar, urea in Urea broth
- Pouring the mixture into Petri plates or test tubes after cooling down
- Labelling the Petri plates/test tubes with the name of the media

While preparing media manually, these steps require the undivided attention of the laboratory personnel. Similarly, these steps may look small but require much more time than expected. Opting for automation in most stages will help the laboratory personnel focus their time and energy on other parts of the diagnosis.

Dissolving the powder

Even though weighing the powder and measuring water is not automated. The manual work of dissolving the powder in water by heating it to the boiling point is tedious and time-consuming. The machines have a powerful magnetic stirrer that completely dissolves the powder. It cuts off the manual labour in half.

Sterilization of the mixture

Autoclaving is one of the ways of sterilizing the dissolved mixture of media, and is a time consuming process with longer waiting time and requires constant supervision by some personnelfor temperature and pressure monitoring. Automated system has provision for heating followed by sterilization in the same equipment without any personnel interference.

Supplement Addition

Some media like blood agar or urea broth needs adding blood and urea after the temperature reaches an appropriate range. Manual operation requires opening of the instrument for such additions and may lead to media contamination. Automated culture media preparation unit has a supplement addition provision (small opening for large volumes; syringe for small volumes) without opening the container's seal.

Dispensing in the Plate

While pouring manually, the volume in each plate maynot be the same, and chances of self-injuries is very high. Automating the pouring/dispensing of media helps obtain an equal volume of media in all the plates. It also helps reduce risk. The rate of dispensation of media in almost 900 plates in an hour. This gives the personnel a walk-away facility.Also, switching between different sizes of the Petri plates is simple. Likewise, filling the test tubes is also accessible in this automation.

Labelling the Plate

It includes naming the prepared petri dishes with all the necessary details of preparation. Automation helps generate barcodes with the date and time of preparation along with the date and name of the media on the Petri plate or any other container. This facility helps in inventory management of the laboratory.

Benefits of Automated Culture Media Preparation and Dispenser System

- Isolating microorganisms becomes hassle-free
- Laboratory requires fewer manual workers
- Contamination-free media
- Well-informed and managed stocks
- Attention to the work that needs manual labor
- Increase in the rate of preparation
- Decrease in the chances of laboratory errors

Automated Culture Media Preparation and Dispenser System
Source: https://www.biomerieux-diagnostics.com/az/

A. AUTOMATED SPECIMEN PROCESSORS

The current generation of specimen processors has far more functionality than manual processing system due to its speed and functionality in proicessing specimens more reproducibly than technicians performing manual plating.

The Selection criteria for selection of a microbiology specimen processing instrument include :were reviewed by Greub and Prod'hom (13). These ey recommended that the following factors be included in the selection of a particular specimen-processing platform: accuracy, capacity, manufacturer's technical support, flexibility (specimen types, loops, inoculation protocols, medium options, laboratory information system [LIS] issues), capacity, flexibility, modularity, and costs (initial costs, costs for any required disposable supplies, and operational labor costs).

The most recent specimen processors include :

Innova(BD Diagnostics, Sparks, MD)

The processor has 5 specimen drawers each holding up to 40 containers(max. 200 containers) (Fig. 2). Specimens can be added as they arrive in a lab with bar coding and with lid/cap intact. Innova uses a universal decapper that decaps/recaps different-sized containers without any manual adjustment. A drawer can hold only a single-sized tube at any one time. There are 6 input stacks with a capacity of 45 plates each (270 plates total). Different agars (including bi-plates) can be loaded into each stack, or all stacks can hold

the same type of agar. The Innova specimen processor includes a full library of traditional streaking patterns; streaked plates are ejected into an output carousel (5 stacks) and can be organized in output stacks by groups so that no sorting is required after streaking. The Innova utilizes reusable 1-, 10-, and 30-µl Nichrome loops. No disposable supplies are required for specimen plating with the Innova.

Fig 2. Innova Processor System

Source: https://www.biomerieux-diagnostics.com/az/

InoqulA FA/MI.

This can be utilized for automated inoculation of liquid specimens and manual plating of other types of specimens (such as wound swabs) as well as for slide preparation (Fig. 3). The streaking process is performed using a magnetic rolling bead, and up to 5 inoculated plates can be struck out at one time, yielding a throughput of up to 400 plates/h. The instrument holds up to 30 types of plated (including bi-plates) and 7 types of tubed media. Inoculated plated media can be sorted in up to 4 different cassettes for different atmospheres of incubation. The manual interaction section of the InoqulA FA/MI instrument permits manual inoculation of non-liquid specimens, such as catheter tips and wound swabs. Once inoculated, these manually inoculated specimens are struck out with magnetic beads, as occurs with liquid-based specimens. A disposable pipette is required for each liquid-based specimen.

Fig 3. InoqulA full automation/manual interaction (FA/MI) specimen-processing device Source: https://www.biomerieux-diagnostics.com/az/

According to studies evaluating the InoqulA processor's inoculation performance, it produced more isolated colonies than hand plating while also demonstrating good repeatability.[8,9,10]

Previ Isola.

The Previ Isola automated plate streaker has 5 different-sized racks, one size for each of 5 different-diameter specimen tubes (Fig. 4). All specimens must be uncapped prior to placing in the instrument. There are 5 input cassettes with a capacity of 30 plates in each stack (150 plates total). Different-agar plates (including bi-plates) can be loaded into each stack, or all stacks can hold the same type of agar. Streaked plates are ejected into output cassettes (3 stacks, 30 plates each) and can be organized by groups so that no sorting is required after streaking. Two different specimen volumes can be inoculated based on plating protocols. A disposable pipette is required for each specimen, and a disposable applicator is required for each plate. The applicator produces a unique radial-comb streak pattern, and there are no other streaking-pattern options. The maximum capacity is 180 plates/h.

Fig 3. PreviIsola automated plate streaker
Source: https://www.biomerieux-diagnostics.com/az/

Studies assessing the inoculation performance of the InoqulA processor have been performed by various researchers worldwide. Chapin et al, reported a 54% decrease in hands-on time using the instrument compared to that for manual planting ($P < 0.0001$) and that the instrument proved much efficient in isolating mixed culture also with high specificity.[11]According to Andrea et al., plating solely urine and preprocessed stool specimens leads in about a $20,000 annual savings in their laboratory.[12]Zimmerman and Trampe tested using saline diluted faeces sample and found that the automatic operation decreased the processing time in comparison to manual culture, and that the Previ Isola process and manual plating were both deemed suitable for 52% and 6% of specimens, respectively.[13]Mischnik et al. evaluated the performance of the Previ Isola instrument on wound specimens with polyurethane swabs in liquid Amies medium in comparison to manually plated wound viscose swabs in Amies medium. They reported that the quality of colony growth on culture media for further investigations was superior with Previ Isola-inoculated plates compared to manual plates.[14]

WASP(Walk-Away Specimen Processor)

It utilizes specimen load and unload conveyors with different-sized pallets for different-diameter tubes (Fig. 4). It uses a universal decapper to automatically decaps/recaps different-sized containers without any manual adjustment. There are 9 medium silos, with a total capacity of 342 to 370 plates (including bi-plates). Each silo or multiple silos can hold a single type of medium. The WASP utilizes two Toshiba selective compliant assembly robot arm (SCARA) robots to move specimens and plates. It includes a full library of streaking patterns, and streaked plates can be organized by groups so that no sorting is required after streaking. Each of two separate cultures can be inoculated to half of a plate and then separately labelled, a practice that is very cost-effective for epidemiological screening cultures. Inoculated plates can be labelled on the side or bottom of the plate. The WASP utilizes reusable 1-, 10-, and 30-µl Nichrome loops with an automatic loop changer. No disposable supplies are required for specimen plating with the WASP. An optional Gram SlidePrep module is available for slide preparation.

Fig 4. The walk-away specimen processor
Source: https://www.biomerieux-diagnostics.com/az/

Bourbeau and Swartz while evaluating the performance characteristics of the WASP reported that no cross-contamination occurs during plating of urine transport tubes and ESwabs and the process is highly reproducible. Additionally, they demonstrated that WASP cultures of Lim broth tubes yielded results that were identical to those of manual culturing.[15]When compared to manually inoculated wound fibre swabs, Jones et al. demonstrated increased detection of Staphylococcus aureus nasal colonisation using ESwabs plated with the WASP.[16]

The factors to consider in the selection of a microbiology specimen processing instrument were reviewed by Greub and Prod'hom (13). They recommended that the following factors be included in the selection of a particular specimen-processing platform: accuracy, capacity, manufacturer's technical support, flexibility (specimen types, loops, inoculation protocols, medium options, laboratory information system [LIS] issues), capacity, flexibility, modularity, and costs (initial costs, costs for any required disposable supplies, and operational labor costs).

Automated Antibiotic Susceptibility Testing

Since the dawn of automated technologies in the 1980s, antibiotic susceptibility tests have been perpetually improvised and, hence, have superseded conventional phenotypic methods (Reller L.B., Weinstein M., Jorgensen J.H., Ferraro M.J. Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices. *Clin. Infect. Dis.* 2009;49:1749–1755. doi: 10.1086/647952.)

Among the developed automated systems, MicroScanWalkAway (Beckman Coulter, Inc. Atlanta, Georgia, USA) (1980), Micronaut (Merlin, berlin, Germany) (1990), the avantage test (Abbott Laboratories, Irving, Texas, USA) (1980), Vitek 2 (bioMe'rieux, Marcy-l'Étoile, France) (2000), Phoenix (BD Diagnostics, Franklin Lakes, New jersey, USA) (2001), and Sensititre ARIS 2X (Trek Diagnostic Systems, Oakwood Village, Ohio, USA) (2004) are the major FDA approved systems for AST. Vitek and Pheonix detect growing bacteria on the basis of turbidity, whereas comparable automated systems like MicroScanWalkAway (Beckman Coulter, Inc. Atlanta, Georgia, USA) and Sensititre ARIS 2X (are based on fluorescence emission of the growing bacteria. The resistance in gram-negative, gram-positive, and STREPTOCOCCUS strains of bacteria can easily be estimated through Phoenix, and Vitek 2, but, MicroScanWalkAway and Sensititre ARIS 2XESBL (Trek Diagnostic Systems, Oakwood Village, Ohio, USA) are capable of detecting the extended-spectrum beta-lactamase (ESBL)-producing strains in the species mentioned above(Sellenriek P., Holmes J., Ferrett R., Drury R., Storch G.A. Comparison of MicroScan Walk-Away®, Phoenix™ and VITEK-TWO® Microbiology systems used in the identification and susceptibility testing of bacteria; Proceedings of the Abstr 105th General Meeting of the American Society for Microbiology; Atlanta, GA, USA. 5–9 June 2005.)Micronaut and Avantage are capable of accurate direct susceptibility testing for gram-positive and gram-negative bacteria, respectively

Genotypic AST Methods

Molecular or genotypic AST are the effective direct methods that eliminate tedious bacterial cultures, long incubation, chances of contamination, and the spreading of deadly infections (Cockerill F.R. Genetic methods for assessing antimicrobial resistance. *Antimicrob. Agents Chemother.* 1999;43:199–212. doi: 10.1128/AAC.43.2.199.)

The most recent molecular methods include :

1. LAMP, which has also been used for the evaluation of AST. In LAMP, the gene of interest is amplified at a constant temperature of 60–65 °C using a BST DNA polymerase instead of TAQ polymerase because of strong strand displacement activity (required in isothermal techniques)(Li Y., Fan P., Zhou S., Zhang L. Loop-mediated isothermal amplification (LAMP): A novel rapid detection platform for pathogens. *Microb. Pathog.* 2017;107:54–61. doi: 10.1016/j.micpath.2017.03.016.)
2. DNA microarrays and DNA chips are the other promising technologies utilized for screening susceptibility(Frye J.G., Lindsey R.L., Rondeau G., Porwollik S., Long F., McClelland M., Jackson C.R., Englen M.D., Meinersmann R.J., Berrang M.E., et al. Development of a DNA microarray to detect antimicrobial resistance genes identified in the National Center for Biotechnology Information database. *Microb. Drug Resist.* 2010;16:9–19. doi: 10.1089/mdr.2009.0082.)DNA arrays employ cDNA fragment probes on nylon membrane, where each DNA chip has a glass or silicon platform for probe binding. The specific hybridization of the labeled probe with the target and its recognition help to determine the resistance. Determination of isoniazid resistance in M. TUBERCULOSIS has been carried out successfully through DNA microarrays and chips(Huang W.L., Hsu Z.J., Chang T.C., Jou R. Rapid and accurate detection of rifampin and isoniazid-resistant Mycobacterium tuberculosis using an oligonucleotide array. *Clin. Microbiol. Infect.* 2014;20:O542–O549. doi: 10.1111/1469-0691.12517.)

Colorimetric detection and multiplexing are the attractive features of these techniques.

Expected Outcome of Automation in Microbiology Laboratories

Bourbeau PP, Ledeboer NA (2013) Automation in clinical microbiology. J ClinMicrobiol 51(6):1658–1665. https://doi.org/10.1128/JCM.00301-13

The expectant rapid changes associated with selection and implementation of microbiology automation solutions will place significant management and financial challenges upon laboratory leadership.

Limitations of automation

- While machines are programmable, humans are more flexible.
- Cost-effective
- Variation in processing so newer machines required
- No logical thinking SO CHANCES OF MISIDENTIFICATION

Conclusion

The choice of a method for the identification of bacteria involves many variables. This article has presented a comprehensive review of the choices available for bacterial identification products along with pertinent information on each one that should aid laboratories in making such decisions. The choice of an identification method depends on a variety of very important factors, some of which a laboratorian may have little control over. The capital resources, workforce technical acumen, physical laboratory size, patient population, and laboratory throughput are the primary driving forces that lead to a final decision. Since the goal of the microbiology laboratory is to provide results that are accurate and clinically relevant, it stands to reason that selecting the identification method contributes to the accuracy component of this paradigm, with the caveat that good microbiologists will use interpretive judgment when accepting a result from any automated instrument. Blind acceptance of species identification without skilled review of potential inaccuracies will eventually lead to misdiagnosis and inappropriate therapy—laboratory errors that we cannot afford to make. Therefore, one must not simply conclude that purchasing an automated identification instrument helps justify employing fewer or less skilled microbiology specialists for any clinical laboratory. Utilizing the information presented in this article, however, should assist management and technologists in the decision process constantly used to optimize our role in infectious disease diagnostics.

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3. **Gram Staining: a Comparison of Two Automated Systems and Manual Staining**

Neele J Froböse[# 1], Sara Bjedov[# 1], Franziska Schuler[1], Barbara C Kahl[1], Stefanie Kampmeier[2], Frieder Schaumburg[3]

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A Literature Review: COVID–19 and post-covid versus Thromboembolism

Mr. Dinesh Kumar Thirugnanam, MSc (Medical Microbiology).,
Tutor, Department of Microbiology
Meenakshi Ammal Dental College and Hospital
Meenakshi Academy of Higher Education and Research
+91 7358873952, E-mail: *dineshthiru1999@gmail.com*

Abstract

In spite of the fact that COVID-19 was previously predominantly believed to be a respiratory ailment, rapidly increasing data point to a significant prevalence of venous thromboembolic consequences in the disease.This review article's main goal was to determine if there was a requirement to raise knowledge of PE (Pulmonary Embolism) in the aftermath of the COVID-19 outbreak given the still-weak epidemiologic data. The gathered studies were subjected to a critical evaluation and literature search.A digital search of Science Direct, Google Scholar, PubMed, and Scopus until June 2022. COVID-19's lasting effects on health are yet mostly unknown. The pathophysiology of pulmonary embolism is highlighted in this review, along with the significance of being aware of the possible ways that enhanced the risk of VTE (Venous Thromboembolism) in patients suffering from post-COVID-19, including those who have a moderate or asymptomatic illness.To define suitable clinical care recommendations for the avoidance of thromboembolic consequences in the critically sick and post-COVID-19 phase, further study is necessary.

Introduction

The recent appearance of SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) has an effect on all of our healthcare systems [1]. Different clinical presentations of the sickness are seen in this condition, COVID-19.The increased thrombotic risk in patients, which may lead to PE and VTE [4–9], is one of the issues with this new condition, COVID-19.On the other hand, there is limited information available on the patient characteristics who present with VTE, COVID-19, and PE. Anticoagulant medication may also improve survival in individuals with severe COVID-19, according to early research [10,11].International associations that

strongly advise the usage of thromboprophylaxis in patients that were hospitalized [12–16] are becoming more and more aware of this problem. Reviewing recent data on the prevalence of PE and VTE (including the COVID-19 outbreak) and evaluating the properties of PE and VTE in COVID-19 were the goals of this research.

Epidemiology

PE is a potentially fatal sign of VTE. In the world each year, 1 in 1000 people experience PE [24,25]. After stroke and myocardial infarction, VTE is the 3rdmost common acute cardiovascular condition worldwide [26,27]. A persistent danger to the general population is COVID-19. The WHO (World Health Organization) has received reports of 770,085,713 confirmed cases and 6,956,173 confirmed fatalities as of June 30, 2023. It is also obvious that PE's frequency has grown throughout the COVID-19 pandemic [28–31].

Database strategy

The lead investigator (DKT) independently conducted a thorough literature search in PubMed utilizing the search terms mentioned here: ('2019-nCoV' OR 'COVID-19' OR 'coronavirus 2019' OR 'SARS-CoV-2'). UNTIL JUNE 30, 2023, AND (thrombosis OR thrombotic OR 'pulmonary embolism' OR 'deep vein'). Articles were also chosen through manual search, journal website searches, and referrals to pertinent articles.

Study Selection

The full-text English papers utilized in the review were eligible research that: (a) registered the occurrence of PE and/or Deep Venous Thrombosis inpatients who aresuffering from COVID-19; and (b) conducted lower limb ultrasonography for DVT screening/assessment in the whole sample or focused on individuals who had PE suspicion.Excluded were case series and case report studies involving less than ten patients. The major objective of the research was to comprehend the etiology of thrombosis in COVID-19 and to estimate the combined prevalence of DVT and PE. The pooled estimate of the odds ratio for mortality in COVID-19 individuals having VTE vs. non-VTE was the study's secondary aim.

Patients

Consecutive patients aged equal to or older than 18 years of age having the confirmation of COVID-19 who is hospitalized for mild to moderate illness (minor clinical symptoms, no evidence of pneumonia on imaging), or to the ICU (Intensive Care Unit) for a severe disease (people with either of the

diseases mentioned here:SPO2 ≤ 93% at rest; respiratory distress with respiratory rate≥30 breaths/minute; PaO2/FiO2≤300mm Hg (1mm Hg=0.133kPa)) to critical (people with either of the diseases mentioned here: shock; failure of the respiratory system necessitating mechanical ventilation; or any otherfailure of the organ) disease, were enrolled. A reverse transcription polymerase chain reaction was performed on sputum samples as well as nasopharyngeal swabs in order to confirm the presence of COVID-19.As per the most recent guidelines for medical patients, all patients were given a prescription for thromboprophylaxis upon admission, either with fondaparinux (2.5mg once a day) or LMWH (low-molecular-weight heparin; 40mg enoxaparin once daily).

Pathogenesis Of Thrombosis In covid–19

A “literature review on the etiology of thrombosis in COVID-19 focuses on the endothelium's important contribution to the hypercoagulable conditionsthat were found in COVID-19 [19–23].ACE2 (Angiotensin-converting enzyme 2) is a transmembrane protein that permits SARS-CoV-2 to enter host cells and reproduce before leaving the cell to infect more host cells, which kills the originating host cell [19, 20].The endothelial cells that line veins and arteries as well as type II pneumocytes express ACE2, the mechanism through which the SARS-CoV-2 infects cells. Angiotensin II, a vasoconstrictor, is changed by ACE2 into angiotensin 1-7 (a vasodilator) during homeostasis, which lowers blood pressure.Whatever the mechanism, ACE2 is internalized after viral entry and subsequently downregulated, which raises the level of circulating (vasoconstricting) angiotensin II. Interferons are released by the host cell in reaction to the viral entrance as part of the innate immune response. This is done in an effort to prevent viral replication in the cell as well as in cells that are nearby. In order to combat the virus, interferons induce the production of pro-inflammatory cytokines which includeTNF-α and IL-1.To stop the spread of the virus, nearby cells are told to go through apoptosis or to destroy the RNA. Additionally produced during apoptosis and host cell death are IL-1 and TNF-α.However, if the virus is effective in producing new virus particles (virions), these virions will leave the cell and may go to the alveolar capillaries to enter circulation. Endothelial cells may now be infected by the virus. The impact of TNF-α and IL-1production on the etiology of thrombosis in COVID-19.Because uninfected endothelium cells that consist of the proinflammatory transcriptional hub nuclear factor-B, cause more of these proinflammatory cytokines to be generated, TNF-α and IL-1 target uninfected endothelial cells.Nuclear factor-κB is stimulated before by angiotensin II expression.Endothelial cells release IL-6 in response to IL-1, and

this molecule works inthe liver for inducing the acute phase response.Macrophages also generate IL-6. As a consequence, the liver produces fibrinogen, CRP (C-reactive protein), and PAI-1 (Plasminogen Activator Inhibitor-1).To create thrombi, fibrinogen, a precursor to fibrin, is utilized. The process that turns plasminogen into plasmin, which causes fibrinolysis, is inhibited by PAI-1. When describing a severe COVID-19 infection, the phrase 'cytokine storm' has been often used.When endothelial cells constantly create IL-1 by inducing the expression of their own genes, the result is a procoagulant condition in the circulation, which is denoted as a cytokine storm in the context of the endothelium.Additionally, IL-1 stimulates the synthesis of TNF- α, which in turn stimulates the synthesis of IL-1.Impaired viral clearance, a lack of type 1 interferons, an abundance of NETs (Neutrophil Extracellular Traps), which are typically antiviral, and viral apoptosis with the subsequent production of proinflammatory cytokines (pyroptosis)are some of the variables that may prevent a cytokine storm in COVID-19.The" endothelium is profibrinolytic and anti-coagulant in nature when the body is in homeostasis. To initiate the coagulation cascade, the subendothelial tissue factor may be activated by viral infection of endothelial cells. Von Willebrand factor is also stored by endothelial cells, where it may be released to promote platelet aggregation and ultimately clot formation. Endothelial cells may produce PAI-1 under the same pro-inflammatory conditions, which prevents fibrinolysis.Due to an imbalance between fibrinolysis and thrombosis brought on by the liver's production of prothrombic acute phase reactants and the procoagulant actions of IL-1 and TNF- α, excessive clot formation results.

Discussion

Thromboembolic PE in post-covid-19 patients

In the last para, we talked about the pathogenetic events that might account for PE at any point throughout the disease or even after the infectious period has passed. These events could take place during any phase of the illness. The late PE may possibly be explained by the protracted COVID's consequences of inflammation, chronic viral replication, hypoxia, as well as endothelial damage leading to organ failure and thrombosis [32].The exact frequency of thromboembolic events during COVID-19 is still unknown, according to a recent meta-analysis, whereas patients who are hospitalized in the ICU are more likely to have a PE [33]. On day 30 after discharge, a different investigation found that the incidence of arterial and venous thrombosis was overall 2.5 percent, while the incidence of VTE alone was 0.6

percent [29]. A “meta-analysis from Kings College Hospital in London found that patients who are hospitalized for COVID-19 don’t have a greater risk of developing thromboembolic disease after discharge compared to individuals who are hospitalized for other acute disorders [30].There is a notable lack of uniformity in the patient population chosen and studied, despite the fact that all studies have a shared purpose in studying the incidence of VTE and the requirement for thromboprophylaxis. It also seems that the information gained from the research differs greatly.According to two more small studies [34,35], the incidence rate of VTE in the initial 30 to 42 days after hospitalization because of COVID-19 is” 0.6 to 0.48 percent.The bed rest is longer, pathophysiological mechanisms, and delayed anticoagulant prophylaxis administration which is involved in the COVID-19 later phases, which are characterized by the interaction among theimmuno-mediated phenomenon, systemic hyper-inflammation state[36], and PE, were all seen to be connected with late hospitalization and PE in COVID-19 patients have been consecutively admitted to 7 Italian hospitals.The majority of PE was diagnosed within 24 hours of admission in the same study, indicating that VTE has not been related to the process of hospitalization. PE in COVID-19 is probably a pathological procedure that progresses over time and begins in the early stages of infection before showing clinical signs in the late infectious stages and necessitating hospitalization [35].Most of the aforementioned research speaks about hospitalized patients who were monitored after being discharged by the hospital or who are now in hospitals.The PE incidence after a moderate, simple, or even an asymptomatic infection of COVID-19, however, is not well understood. In order to examine the patients, the progression of their condition when it is positive for COVID-19, along with thepresentation with VTE at the time of post-COVID-19 more closely, our goal with this research was to compile all the available literature data.The majority of the time, the 1stmonth after COVID-19 infection, is when PE's main symptoms occur. The majority of patients did not get anticoagulant therapy, mostly because their infections only caused moderate symptoms in them. The instances roughly equally include people who are in their fourth, fifth, and sixth decades of life.We require further study and/or registries to have a firm understanding of the PE incidence following infection of COVID-19 and the period of time when there is an elevated PE risk, even if a tendency may be characterized with the help of case studies. In fact, it raises the issue of whether we should include theinfection of COVID-19 as a separate risk factor when estimating the likelihood that PE would emerge.

Conclusion

Despite receiving thromboprophylaxis in the majority of instances, our evaluation of the literature indicates that hospitalized COVID-19 patients who are tested or evaluated for VTE have a pooled occurrence of PE &DVT at roughly 30 percent each. Patients with COVID-19 hospitalized in ICUs and who have recovered from the infection of COVID-19 seem to be at much increased risk for developing PE. Further study is required to determine the ideal preventive anticoagulant medication, underlying pathogenetic processes, and the particular PE and VTE risk of individuals with COVID-19 infection.

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Chronobiology, Chronopharmacology and Drug Delivery

Dr.Manjusha Pouranik
Associate Professor
Mata Gujri Mahila Mahavidyalaya, Jabalpur, Madhya Pradesh
E-mail: *drmanjusha123@gmail.com*

Introduction

The study of periodic (cyclic) events in living things and how they adjust to the cycles of the sun and moon is known as chronobiology. Biological rhythms are the name given to these cycles. "Chrono" refers to time, while "biology" is the study of life or the science of it. The names chronomics and chronome, which are related, have occasionally been used to characterize the molecular processes underlying chronobiological phenomena or its more quantitative facets, particularly when it comes to comparing the cycles of different organisms.Comparative anatomy, physiology, genetics, molecular biology, and organismal behaviour within biological rhythm mechanics are few topics that covered in chronobiological studies.

Chronopharmacology, a relatively young field of pharmacy (the science and technology of drug dosage forms), can effectively treat illnesses. The field of chronopharmacology describes how a treatment's impact alters over time and with endogenous regularity.

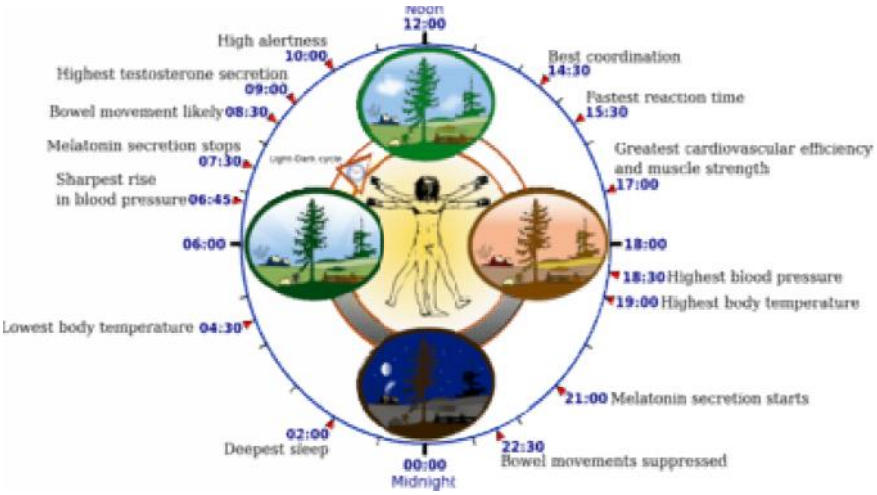
The subspecialties of chronopharmcology are chronopharmacotherapy, chronopharmacokinetics, and chronotoxity.Using the fundamental ideas of human chronobiology, the rhythm dependence of specific disease states, and the pharmacodynamics of medications, chronopharmaceutics is a subfield of pharmaceutics dedicated to the design and evaluation of drug delivery systems that release a bioactive agent at a rhythm that optimally matches in real time the biological system.

Biological Rhythms

For many crucial biological processes, there are differences in the time and duration of biological activity in living things.These take place in both (a) plants and (b) animals, including (animals (feeding,sleeping, mating, hibernation, migratory, cellular regeneration, etc.).

Circadian Rhythm

The most significant rhythm in chronobiology is the circadian rhythm, which is an approximately 24-hour cycle revealed by physiological processes in both plants and animals. The word circadian is derived from the Latin words circa, which means "around," and dies, which means "day," which means "roughly a day." Both (a) in animals (such as rnating, migration, and cellular regeneration) and (b) in plants (such as leaf motions and photosynthetic reactions) are researched. Fig1



Circadian Cycle credit:Wikipedia

Infradian rhythms

These long-term cycles include the menstrual cycle in women and the annual migration or reproductive cycles found in some animals.

Ultradian rhythms

Ultradian rhythms are brief cycles like the 3-hour growth hormone production cycle, the 4-hour nasal cycle, and the 90-minute REM cycle. They have cycles that are shorter than 24 hours.

Tidal rhythms

Tidal rhythms are frequently seen in marine life and are a result of the roughly 12-hour cycle from high tide to low tide and again.

Clock Genes

As a result of cyclic oscillations in the expression of a network of multiple "clock genes," many human tissues have their own internal clocks. Derk-Jan Dijk, director of the Surrey Sleep Research Centre in Guildford, United Kingdom, asserts that "the entire body is a clock." There are clocks in every room and corner of the home, but they all function in a coordinated manner. The timing of all these different "peripheral oscillators" has a significant impact on several important processes, including metabolic activity and immune cell proliferation. But the suprachiasmatic nucleus (SCN), a collection of neurons in the hypothalamus, serves as a central pacemaker that informs the body of the time of day. Fig 2

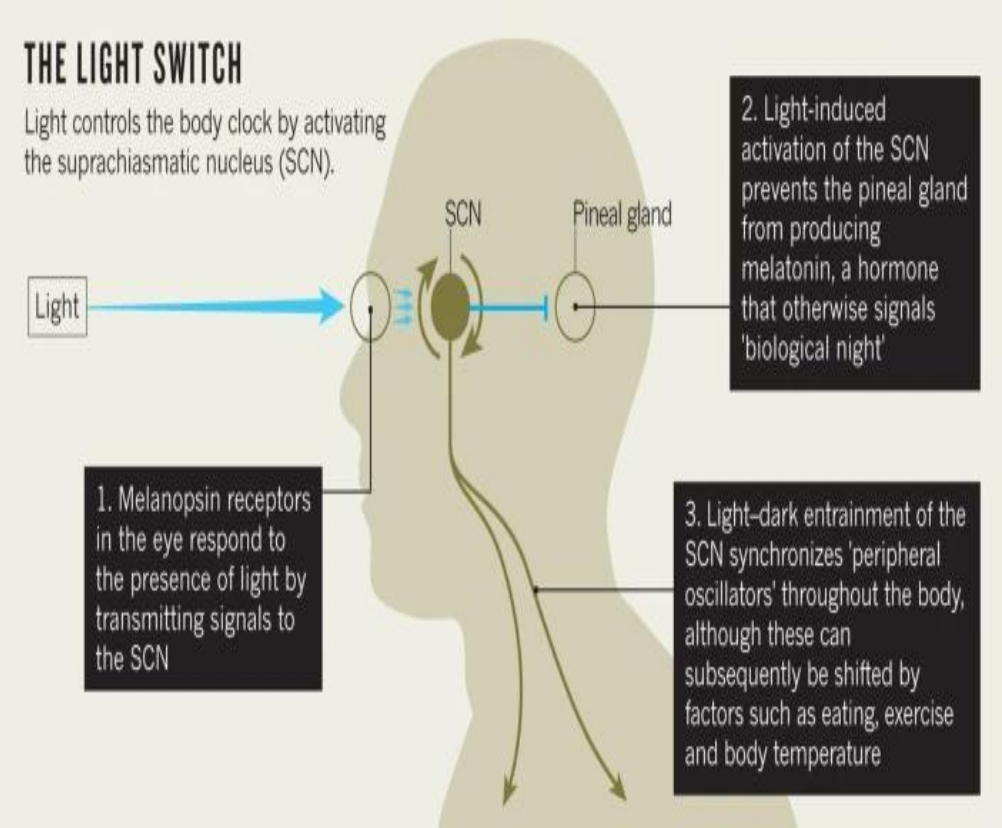


Fig 2. Credit:Eisenstein, M. Chronobiology: Stepping out of time. *Nature* 497, S10–S12 (2013). https://doi.org/10.1038/497S10a

The SCN is activated when the melanopsin photoreceptors in the eye detect light. In response, the SCN starts a number of physiological processes that create rhythms, such as preventing the pineal gland from producing the hormone melatonin. Peripheral oscillators can be impacted by physical activity and dietary changes, but most research identifies light exposure as the single most important factor affecting rhythms regulated by the SCN. "If you look at the data for humans, every time they suggested that food or exercise may have shifted the clock, they also suggested that light may have been involved," says Debra Skene, a chronobiologist at the University of Surrey in the United Kingdom.

Innate Instincts

Humans are a diurnal species, which means we spend the day working and the night sleeping. Individual sleep preferences, or whether a person is an early bird or a night owl, can, however, fluctuate substantially between persons and even between different stages of life.

Chronopharmacology:

By scheduling drugs in respect to biological rhythms, this study seeks to maximize pharmacological benefit while minimizing side effects. The objective is to enhance our comprehension of periodic and hence predictable variations in both desired effects and pharmaceutical tolerance. The branch of biology that studies how drugs affect biological rhythms is known as chronopharmacology.It is well known that certain illness situations cause specific body activities to be dependent on the circadian rhythm. The brain releases some hormones in the morning while other hormones are released as you sleep.Both the illness status and the plasma medication concentrations are affected by these variations. The human circadian rhythm, which is based on the sleep-activity cycle and is controlled by genetics, affects how the body performs during the day and at night affects the body’s functions day and night (24-hour period) [2]. The dependence of bodily functions in certain disease states on circadian rhythm is well known.For example The hours from six in the morning until noon are when blood pressure and heart rate are at their maximum [3].The body's circadian rhythm is followed by diseases like hypertension, asthma, peptic ulcer, arthritis, and others [4].While rheumatoid arthritis patients typically have pain that peaks in the morning and gradually subsides during the day, osteoarthritis increases throughout the day and is most troublesome in the evenings. Chest discomfort and other cardiovascular conditions like hypertension and angina exhibit a distinct circadian pattern.Myocardial infarction, stroke, and angina are all more common in the

morning, according to epidemiologic studies [5].The significance of biological rhythms in medication therapy has been shown by research in the chrono pharmacological discipline, and this has led to a novel method of developing drug delivery systems. This may help a medication work more effectively or have fewer unfavorable side effects. Contrary to the conventional homeostatic strategy, the risk of errors and/or incorrect information is decreased using chronopharmacologic procedures. The potency and toxicity of numerous pharmaceuticals vary in accordance with the dosing regimen that matches the 24-hour rhythm of the biochemical, physiological, and behavioral processes that are controlled by the circadian clock. Both the pharmacodynamics and the pharmacokinetics of the drug have an effect on such chronopharmacological occurrences. The significance of biological cycles in medication therapy has been shown by research in the chronopharmacological discipline, and this has led to a novel method of developing drug delivery systems. Constant drug plasma concentrations are incompatible with achieving the best possible clinical results. If a disease's symptoms vary according to the circadian rhythm, medicine release need to too. In recent years, there has been an increase in the use of various technologies to create time-controlled, pulsed, triggered, and programmed drug delivery systems.

ChronopharmacologicApproaches in Different Diseases

Continuous monitoring of blood pressure throughout the day and night reveals a pattern with minimum values of systolic & diastolic pressure between midnight & 4 am. Early in the morning B.P begins to rise from the low levels reached during sleep. Increases in blood pressure are accompanied by increase in heart rate caused by the chemical generated by the body & delivered into the blood stream.The treatment of numerous cardiovascular disorders, including hypertension, myocardial infarction, angina pectoris, pulmonary embolism, etc., now makes use of chronopharmacological principles. The danger of errors and/or erroneous information is lower with chronopharmacologic techniques than with the traditional homeostatic approach. Numerous medications' potency and toxicity fluctuate according to the dosage schedule that corresponds to the 24-hour rhythm of the biochemical, physiological, and behavioral processes that are regulated by the circadian clock. Such chronopharmacological events are impacted by the pharmacodynamics as well as the pharmacokinetics of the drug.By adjusting the dosing schedule depending on the chrono-biological rhythm, the pharmacological therapy can be made as effective as possible.

Compared to the traditional homeostatic technique, chronopharmacologic approaches carry a lower probability of errors and/or incorrect information. The 24 hour rhythm of biochemical, physiological, and behavioral processes under the control of the circadian clock affects the effectiveness and toxicity of many medications. Such chronopharmacological phenomena are affected by both the pharmacodynamics and pharmacokinetics of the drug.By customizing the dose schedule based on chrono-biological rhythm, the pharmacological therapy can be improved.

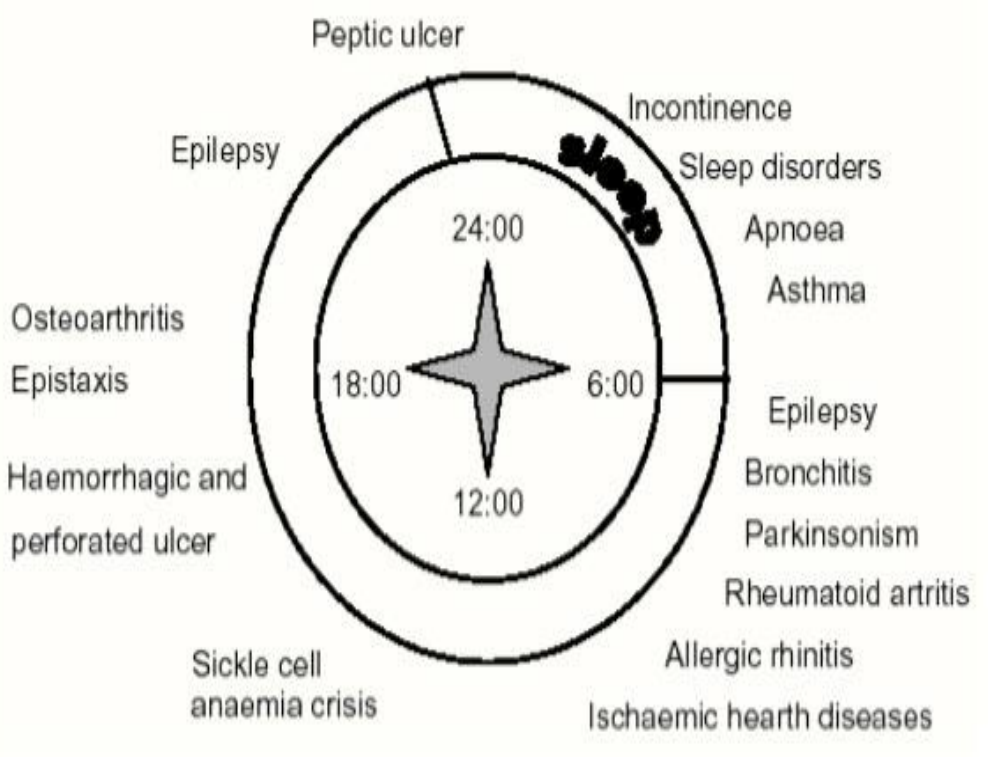


Fig 4 Credit:CHRONOPHARMACOLOGY: AN OVERVIEW

Chrono-Pharmaceutics

The science and invention of medication measurement shapes) that focuses on the design and assessment of drug delivery systems that release a bioactive specialist at a state that, in an ideal world, corresponds to the physiological requirement for the treatment of a given disorder or continuous counteractive action. Chrono-pharmaceutics is one such branch of pharmaceutics.

The Chronopharmaceutics Drug Delivery System is based on the fundamental principles of human chronobiology, the pharmacodynamics of prescription medications, and specific clinical conditions. 6. Chrono-pharmacokinetics refers to the dosing time dependence on rhythm fluctuations in parameters used to describe the pharmacokinetics of medication. 7. The chronopharmacokinetics of a particular medicine may involve changes from a mono- to a multi-compartmental model as a component of drug dose time.

Chronotherapeutics

Basic chronobiological research that has been transformed into effective therapies is known as chronotherapeutics. Since the term is so general, treatments that fall under this umbrella are not just for emotional disorders.In order to maximize effectiveness and reduce toxicity and negative effects, chronotherapeutics is the practice of administering treatment or intervention with regard to circadian rhythms. Since the human circadian rhythm also regulates a variety of additional physiological and psychological functions, such as hormone synthesis, sleep patterns, behavior, and metabolism. Endogenous biological rhythm has a direct impact on the kinetics and dynamics of medication, and drug administration timing has an impact on biological timekeeping and biological rhythmic features (period, level, amplitude, and phase). Internal factors like aging, heredity, and other factors can create a long-lasting disruption of the circadian rhythm. Nearly half of all genes exhibit circadian oscillations in transcription in one or more tissues in the body, highlighting the importance of chrono pharmacodynamics (chronoPD), which specifically considers how drug pathways will be affected by the rhythms of its targets as well as the rhythms of physiological proscessvmodulating the extracellular environment. In addition, in order to identify an optimal drug–target inter. An important aspect of chrono pharmacodynamics (chronoPD), which specifically considers how drug pathways will be affected by the rhythms of its targets as well as the rhythms of physiological processes modulating the extracellular environment, is the fact that nearly half of all genes exhibit circadian oscillations in transcription in one or more tissues in the body. Chronopharmacokinetics (chronoPK) investigates how physiological cycles affect the absorption, distribution, metabolism, and elimination of medications, contributing to changes in possible adverse effects across the circadian cycle, in order to establish an ideal drug-target interaction.An ideal therapeutic window within the circadian cycle might be found when these elements are taken into account along with the drug's half-life .

Chronopharmacokinetics (chronoPK) investigates how changes in physiological rhythms across the circadian cycle impact medication absorption, distribution, metabolism, and elimination, potentially changing their potential adverse consequences.An ideal therapeutic window within the circadian cycle might be found when these elements are taken into account along with the drug's half-life.

Drug Delivery

The goal in drug delivery research is to develop formulations to meet therapeutic needs relating to particular pathological conditions. Optimal clinical outcomes cannot be achieved if drug plasma concentrations are constant. If symptoms of a disease display circadian variation, drug release should also vary with time. Utilization of different technologies in the development of time-controlled, pulsed, triggered and programmed drug delivery devices has intensified in recent years.Worldwide several researches are going on for the development of new drug delivery systems. In conventional therapy drugs are released immediately after medication. So, the drug concentration in the plasma is raised and sometimes it is more than the toxic level. The target of drug discovery is to obtain maximum drug efficacy and minimum side effects. With the advancement of technologies in the pharmaceutical field drug therapy has changed its path. Although sustained and constant release systems have been developed, biological systems are not so responsive to these release systems.Fig 5

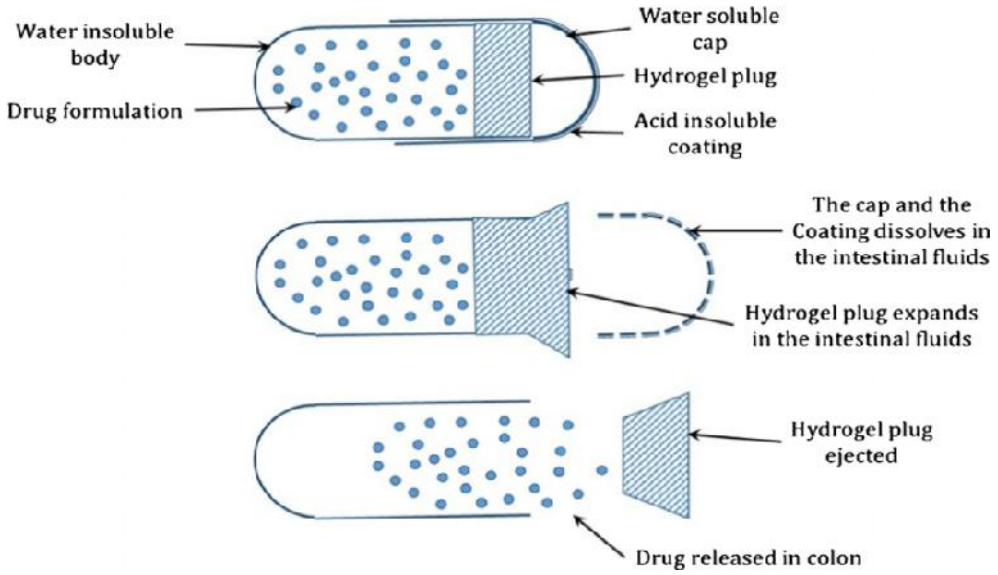


Fig 5 Credit: Research gate

The release of some drugs is preferred in pulses i.e. the release of a drug as a "pulse" after a lag time has to be designed in such a way that a complete and rapid drug release should follow the lag time. (9) Lag time is defined as the time between when a dosage form is placed into an aqueous environment and the time at which the active ingredient begins to release from the dosage form. (Fig6). These systems are also called time-controlled as the drug released is independent of the environment (10). A single dosage form provides an initial dose of drug followed by one release-free interval, after which a second dose of drug is released, which is followed by additional release-free interval and pulse of drug release.

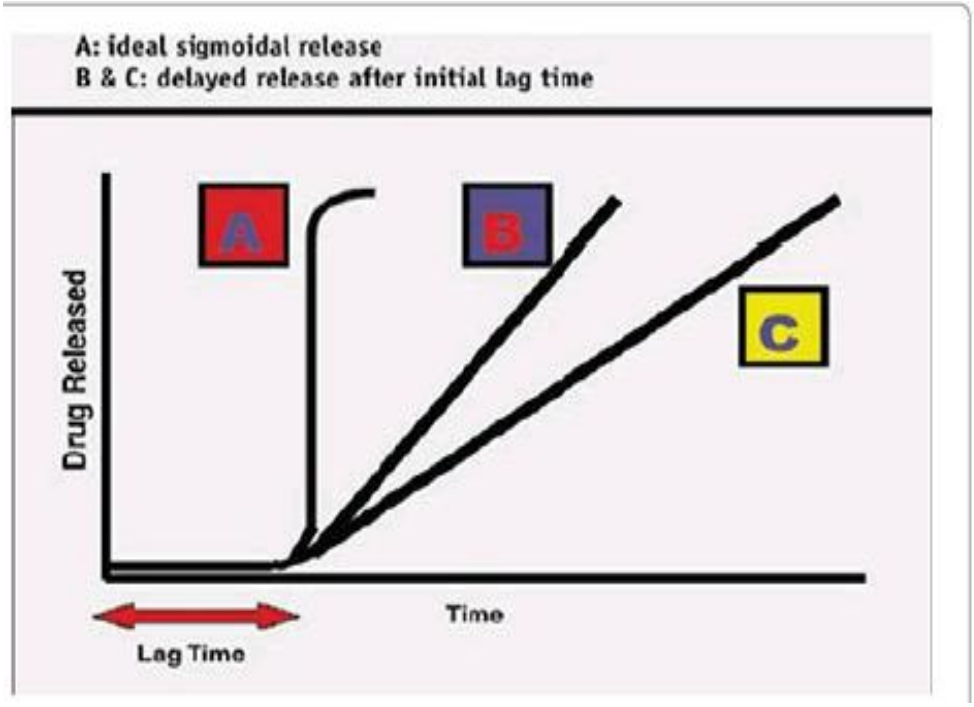


Fig 6 Drug delivery system

Conclusion

Prior to now, it was challenging to cure disorders brought on by a biological clock that was out of balance. These illnesses fell beyond of pharmacology's purview. However, after this clock was understood, it became possible to create medications through the study of the chronopharmacodynamics of specific chemical substances and treat such illnesses to provide patients with some comfort.The development of drug delivery systems aimed at the treatment of diseases with an adequate dose at

the proper time must therefore take into account variations in disease status and drug plasma concentration. [12,13].

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Dental pulp stem cells and its applications in regenerative medicine

Yogavathi P S[1], Gayathri S[2] and Deepa Rajendiran[3*]
[1, 2] Second Year BDS, Department of Biochemistry,
Madha Dental College and Hospital, Chennai, Tamil Nadu, India
[3*] Associate Professor, Department of Biochemistry,
Madha Dental College and Hospital, Chennai, Tamil Nadu, India.
E-mail: *deepanish1981@gmail.com*

Abstract

Stem cells are undifferentiated cells with the capacity to differentiate into multiple cell types.The use of adult stem cells is of particular interest when it comes to dynamic applications in translational medicine. Dental pulp stem cells (DPSC) are one of the most promising sources of stem cells for tissue engineering and regeneration. This review article mainly focuses on the isolation procedure, cultural conditions, cryopreservation, various properties and clinical applications of human derived dental pulpal stem cells (DPSCs) in regenerative medicine

Introduction

Stem cells are specialised and undifferentiated cells that can multiply into different cell types. Embryonic stem cells and adult stem cells are the common types of stem cells. Adult stem cells were first isolated from bone marrow, later they were isolated from different organs from skin, adipose tissue and vascular endothelial cells.In a variety of somatic illnesses, MSCs (mesenchymal stem cells) are anticipated to promote tissue regeneration.

MSC-like populations have also recently been found in the oral tissues. Dental pulp stem cells (DPSCs) were initially found by Gronthos *et al*from human pulp tissue. In addition to stem cells, fibroblastic cells, capillary blood vessels, peripheral nerves, lymphatic components, extracellular matrices, and odontoblasts in the pulp tissue's periphery make up dental pulp, which is an unmineralized connective tissue. DPSCs are cells with strong clonogenicity, proliferative activity, and the capacity to form mineralized nodules. They have been isolated from adult human pulp tissue of impacted third molars, orthodontic teeth, and supernumerary teeth[1]. They are proposed to be

prospective cell sources for the regeneration or restoration of numerous somatic tissues, including those in the cranio-maxillofacial area, because of their MSC-like traits. Consequently, stem cell biology, which uses these cells, has emerged as a crucial area of study for comprehencing and applying regenerative medicine.

This review aims to summarize the features of DPSC's and their potential to regenerate damaged tissue as well as prospects of cell reprogramming applications in regenerative applications.

Stem cells in the tooth compartments

Tooth compartments, like other organs, have a diverse variety of stem/progenitor cell populations of embryonic stem cell[2]. These cells regenerate the dentin barrier to shield the pulp from infectious agents and exhibit an immunomodulatory capacity, according to several studies. During the post-inflammatory recovery phase of minor dental trauma, they accomplish this via secreting proinflammatory cytokines or interacting with immune cells[37]. The various sources of dental progenitor cells include) the dental pulp of permanent teeth (DPSCs)[1]) the naturally exfoliated deciduous teeth (stem cells from human exfoliated deciduous teeth (SHED))[8]) the periodontal ligament (periodontal ligament stem cells (PDLSCs))[9]) the dental follicle (dental follicle progenitor cells (DFPCs))[10]) the apical papilla (stem cells from apical papilla (SCAP))[11] and) the oral mucosa and gingiva (gingiva-derived mesenchymal stem/stromal cells (GMSCs))[12]. Figure 1 represents the sources of oral tissue and dental stem cells.

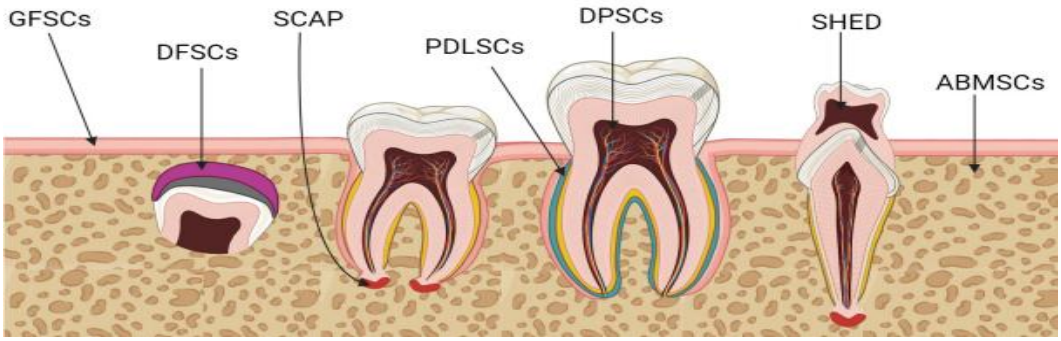

Figure 1: Sources of Oral tissue and Dental stem cells

Isolation procedure and culture methods

Permanent and deciduous (primary) teeth considered an easily accessible source for isolation of dental pulp cells. In the earliest study, DPSCs are isolated from permanent teeth of healthy adult participants, aged 18 to 85 years, when sound third molars are extracted. The criteria for deciduous teeth are healthy children; 9 to 12 years old, with teeth near to physiological tooth exfoliation. Dental pulp stem cells are cultured following an optimal temperature and environmental incubation period. Cells were either employed for an experiment or cryopreserved for subsequent use after they reached 70 to 80% confluency.

Cells were properly washed twice with 1X PBS (phosphate-buffered saline) before being trichinized for two to five minutes with 0.05% trypsin-EDTA (ethylene diamine tetra acetic acid).The supernatant must be carefully decanted, without disturbing the cells.Cells were either frozen using media containing 90% FBS (fetal bovine serum) and 10% DMSO (dimethyl sulfoxide) in liquid nitrogen at -196°C for subsequent use or long-term storage[13].

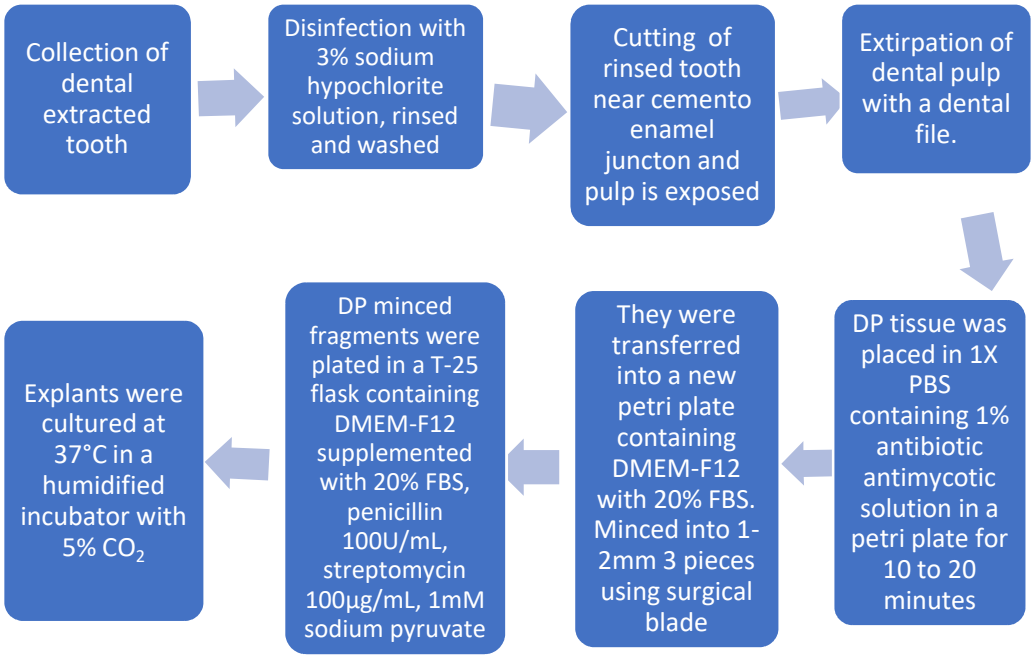

Figure 2: Isolation and culture methods of DPSCs.

Properties of human dental pulp derived stem cells (HDPSC)

The HDPSCs have characteristics in common with mesenchymal stem cells. They may sustain long-term self-renewal due to their undifferentiated lineage. They also can develop into progenitor cells. They can differentiate into mesodermal, ectodermal, endodermal, osteogenic, chondrogenic, and adipogenic lineages[14].

1. Self-renewal

One of the fundamental characteristics that distinguishes stem cells is their capacity for self-renewal. According to self-renew Capacity, it is divided into two categories.Asymmetric division creates both undifferentiated cells and cells destined to differentiate, whereas symmetric division produces daughter cells with developmental potential similar to that of the parent stem cells(Figure 3)[15].

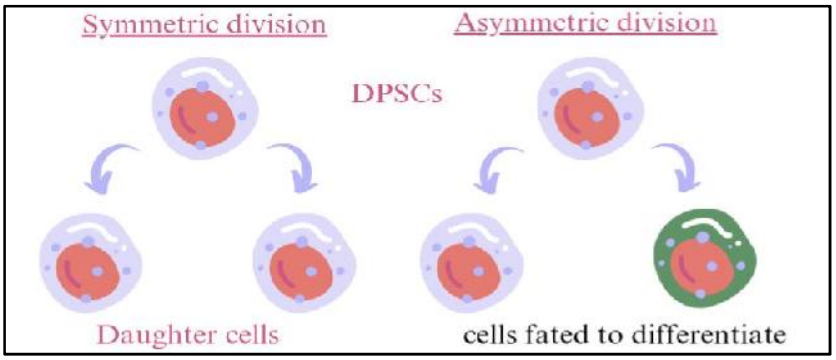

Figure 3: Self renewal capacity of DPSCs.

2. High proliferation activity

Mesenchymal stem cells (MSCs) generated from bone marrow (BMMSCs) are highly capable of self-renewal, making them an attractive cell source for cell-based therapeutics, drug development, and biomedical research. In a prior work, human BMMSCs generated from aspirated bone marrow cells of adults (20-35 years of age) were compared to human DPSCs isolated from extracted third molars of adults (19-29 years of age) in terms of colony-forming efficiency and cell proliferation[1]. Surprisingly, compared to human BMMSCs, human DPSCs displayed higher frequencies of colony-forming cells and more proliferating cells. Due to their high telomerase activity, human

DPSCs would keep their telomeres at lengths larger than those of human BMMSCs.

3. Multipotency of DPSCs

DPSCs can differentiate into various cell types under appropriate culture conditions. Stem cell technology enables to induce HDPSCs into ectodermal lineage cells such as neural cells; mesenchymal lineage cells such as odontoblasts, osteoblasts, chondrocytes, adipocytes, and myocytes[16], endodermal lineage cells such as vascular endothelial cells, hepatocytes and pancreatic islet-insulin-producing β cells[17].

In recent studies, DPSCs have also been proven to differentiate into cardiomyocyte-like cells and corneal epithelial cells[18-26]. It also been proven to differentiate into cardiomyocyte-like cells when they were co-cultured with neonatal rat cardiomyocytes in vitro [27,28].

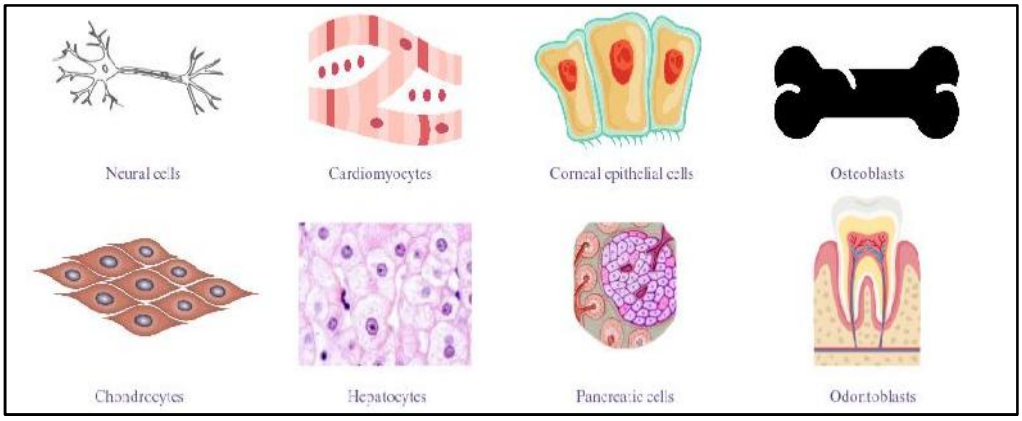

Figure 4: Differentiating potential of DPSCs.

4. Cell Markers Expression in DPSCs

DPSCs express MSC-related markers, such as CD13, CD44, CD73, CD90, CD146, CD166, and STRO-1[30-39].Similar to ES cells, it has been demonstrated thatDPSCs also exhibit pluripotency markers such as Oct-4, Nanog, Sox-2, and the insulin-like growth factor 1 receptor (IGF1R)[40,41].

Table 1: Represents cell markers expression in DPSCs.

Marker Type	Expression Markers	DPSCs	Reference
MSC-related	CD13, CD44, CD73, CD90, CD146, CD166, STRO-1	+	Gronthos, *et al*., 2000 Miura, *et al*., 2003 Govindasamy, *et al*., 2010
Osteogenic	BMP2, OCN, OPN, Osteonectin, Col-1	+	Karaoz, *et al*.,2010 Patelet al., 2009
Neurogenic	Nestin, GFAP, β- tubulin, MAP-2	+	Gronthos, *et al*., 2002 Vishwanath *et al*., 2013
Pluripotent	Oct-4, Nanog, Sox2, IGF1R	+	Kaukuaet al., 2015 Karaoz, *et al*., 2010

5. Immunomodulatory Effects of DPSCs

DPSCs have immunomodulatory effects through regulating the proliferation and cytokine generation of immune cells.To control the activation of immune cells, DPSCs also express immunomodulatory and anti-inflammatory substances on their own.Co-culture cell models have revealed that DPSCs mediate G0/G1 cell cycle arrest of the chemically-activated T cells[42]while, other studies also show induction of differential T-cell subset responses. Co-cultures of DPSCs with CD3+, CD4+, or CD8+ T cells induced regulatory T cells (Treg) and/or differentially arrested growth[43-46].

6. Regenerative Capacity of DPSCs

The most notable feature of DPSCs is their odontoblastic differentiation ability. The full regeneration of the dentin/pulp complex, including tubular dentin development, pulp innervation, pulp immunity, and pulp vascularization, is the aim of endodontic treatment.

6.1. Regeneration of Dentin/Pulp Complex and Other Dental Tissues

In immunocompromised mice, DPSCs combined with hydroxyapatite/tricalcium phosphate (HA/TCP) or nanofibrous poly-L-lactic acid (PLLA) were subcutaneously implanted, where they developed into odontoblasts and produced dentin with vascularized pulp-like tissue[47-51].

To further understand how DPSCs and SHEDs affect the regeneration of periodontal tissue, numerous investigations have been carried out. For

instance, in a canine periodontitis model, Khorsand et al[52] transplanted DPSCs with bone granules into periodontal defects and found that the DPSCs formed cementum and periodontal ligament (PDL) tissue. Additionally, Janebodin *et al.* showed that when combined and subcutaneously implanted into immunocompromised mice, DPSCs improved the development of human salivary gland (HSG) cells into functional salivary gland tissue[53].

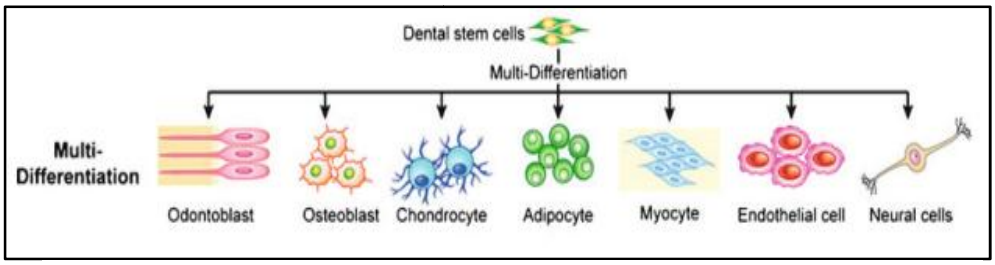

Figure 5: Differentiation potential of DPSCs.

6.2 Regeneration of Other Somatic Tissues

DPSCs have been reported to possess the ability to regenerate or repair various somatic disorders such as cornea trauma[54], glaucoma[55], muscular dystrophy[56], acute myocardial infarction[57], spinal cord injury [58], liver fibrosis[59], cerebral ischemia[60], diabetic neuropathy[61], and osteoporosis[62].

6.3 Cell-free Methods for Regenerative Medicine

Exosomes produced from DPSCs enhanced DPSC odontogenic differentiation and aided in the regeneration of dental pulp-like tissue [63, 64].

7. Differentiation Potential of Dental Pulp Stem Cells:

Another essential characteristic of stem cells and DPSCs is their capacity for differentiation. According to Yamada *et al*[65], DPSCs can differentiate into three separate lineages: ectodermal (skin and neural lineages), mesodermal (adipogenic, osteogenic, and chondrogenic lineages), and endodermal (respiratory and gastrointestinal tracts, liver, pancreas, thyroid, prostate, and bladder lineages).In another study the differentiation of DPSCs into myocytes, cardiomyocytes, hepatocyte-like cells, melanocytes, and active neurons was also demonstrated[66,67.] (Figure 5).

Clinical application

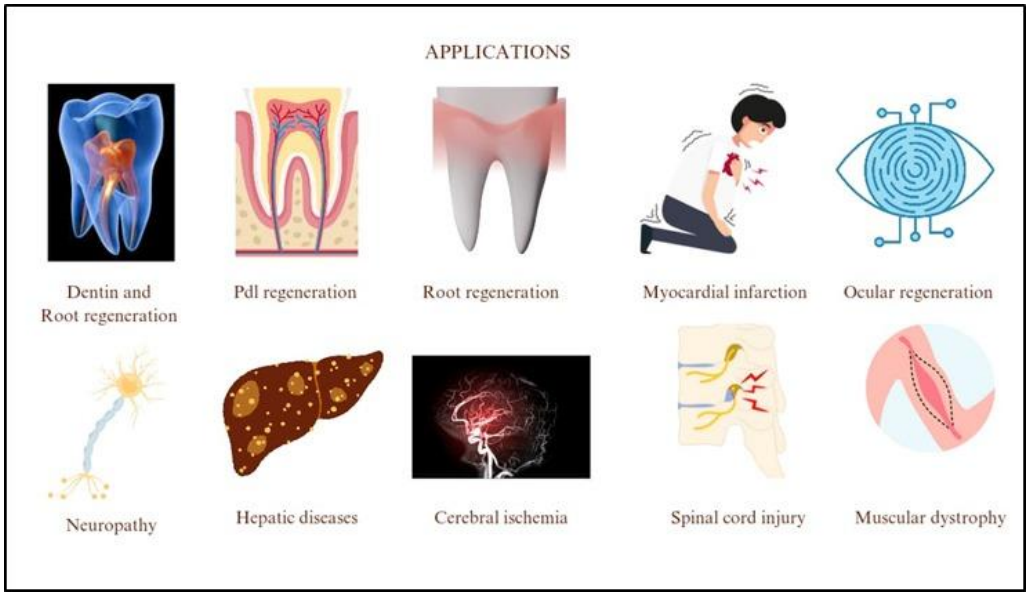

Figure 6: Clinical applications of DPSCs.

The numerous uses of HDPSCs are based on ability to regenerate tissue, their multipotency, and immunomodulatory properties.The major applications are as follows

Periodontal tissue regeneration

To cure periodontal diseases, Kawaguchi et al. used BMSCs because of their capacity to form alveolar bone, PDL, and in vivo cementum that follows implantation into periodontal abnormalities[68]. Using autologous bone marrow stem cells and scaffold, Mary et al. succeeded in regenerating periodontal tissue around titanium implants in an experiment on a goat[69]. It has been demonstrated that periodontal tissue can regenerate when PDL-derived cells are implanted into animal models[70]. Primary canine PDL cells were isolated, multiplied in vitro, and converted into transplantable constructs using PGA(polyglycolic acid) scaffold and PDL cell sheets by Iwata *et al*.Alveolar bone, cementum, and periodontal fibers were all regenerated by the transplantable constructs in conjunction with porous b-tricalcium phosphate[71].

Dentin/Pulp tissue Regeneration

Vascularization provides the foundation for the regeneration of the pulp complex of the dentin. Managing the vascular endothelial growth factor promotes vascularization, but has a shorter half-life. Binding with heparin makes it possible to increase this. When stem cells are treated under hypoxic conditions, vascularizing substances are secreted by the cells.To govern stem cells, which develop into different types of cells, growth factors like the soluble protein of the dentin matrix must be used.The removed tooth that underwent root canal therapy was filled with DPSCs and then implanted on the dorsal surface of immune-compromised mice. Blood vessels are found in the regenerated dentin, which are located close to the pre-existing dentin and beneath the de novo dentin in the connective tissues[72].

The dentin-pulp complex is being clinically tried to rejuvenate via autologous transplantation of DPSCs. When human pulp stem cells with scaffold (HA/tricalcium phosphate) were implanted in immunocompromised mice, tubular dentin development was seen. In experimental research using animal models, stem cells and recombinant human bone morphogenetic protein 2 were found to promote the production of reparative dentin on severed pulp[73].

Bone regeneration

In the previous studies several chemical compounds have been used to induce bone formation. TH [4-(4-methoxyphenyl)pyrido[40,30:4,5]thieno[2,3-b] pyridine-2-carboxamide], a Helio xanthin derivative, induces osteogenic differentiation of pre-osteoblastic and mesenchymal cells[74]in vitro and in vivo[75-77].According to Lymperi*et al*., placing a bio complex of collagen sponge filled with DPSCs in the extracted site of the third molar on the mandible led to a greater rate of mineralization and cortical levels, which ultimately led to complete regeneration. The samples also revealed a lamellar bone structure surrounding the Haversian canal that was well-organized and vascularized. They also show promise in the management of degenerative conditions affecting the mandible and maxilla[78].

Central nervous system

Exogenous stem cells (DPSCs) promote neural and glial growth as well as regeneration of new neural progenitor cells. They can lead to the survival and maintenance of existing neural cells by secreting trophic factors [79-80].

Stroke

Neurons subject to ischemia are unable to maintain a normal transmembrane ion gradient and balance, which leads to apoptosis, excitatory toxicity, and oxidative stress, which all cause cell death. According to Sowa *et al*[81]., the delivery of DPSCs during the acute phase of stroke reduces inflammation in vivo and can speed up recovery from post-ischemia/reperfusion brain injury. Furthermore, intracerebral transplantation of DPSCs or immune-sorted IGF1R+ dramatically enhances poststroke recovery of the chemically damaged neonatal murine brain, promotes neuroplasticity and increases immunomodulation[82,83].

Further, intravenous transplantation of DPSCs or DPSC-derived neurosphere cells significantly ameliorates the impact of global cerebral ischemia, decreases neuronal cell death in the hippocampal CA1(cornu ammonis) region, improves neuromotor and cognitive function as well as overall survival rates in stroke animals[84]. Moreover, intracerebral transplantation of DPSCs also enhanced poststroke functional recovery after brain injury through increasing expression of the anti-apoptotic protein Bcl-2(B cell lymphoma).

Muscular:

In diseases like muscular dystrophy, DPSCs can develop into multinucleated muscle cells that produce dystrophin. When dental pulp myogenic progenitor cells were used instead of the diverse stem cells present, more dystrophin was generated[85].

Diabetes

Diabetes is a chronic degenerative disease. Pancreatic islet cells transplantation is also a treatment for diabetes. Chen et al. demonstrated that insulin-producing cells can be derived from monoclonal and polyclonal DPSCs[86]. Govindasamy *et al*. demonstrated that DPSCs have the capacity to differentiate into islet-like aggregates[87].

Advantages and challenges

Dental pulp stem cells (DPSCs) may regenerate a dentin/pulp-like complex and have a greater ability to differentiate. The differential ability of DPSCs has been demonstrated in numerous studies, including neurogenesis, adipogenesis, osteogenesis, chondrogenesis, angiogenesis, and dentinogenetic. Ischemic stroke is a leading cause of disability and mortality globally, however there are currently very few effective restorative therapies available. Dental

pulp stem cells (DPSCs) are autologously applicable cells with neuro-ectodermal characteristics along with multilineage differentiation potentials that develop from the neural crest. This is the main benefit of DPSCs over other forms of stem cells.Although challenges remain, including the mechanisms of action and establishing cell processing and transplantation methods for clinical purposes, DPSCs may be a promising source of stem cells for clinical applications and they are easily isolated by a non-invasive process without ethical concerns.

Conclusion

DPSCs are a potential cellular source for regenerative medicine applications as well as for cutting-edge stem cell research. The fact that DPSCs originate from the neural crest during embryonic development distinguishes them from other adult stem cell types. However, further research is needed to understand the processes behind the therapeutic effects of DPSCs. The future therapeutic technique will be regenerative; however, additional research with long-term follow-up is required to test the various possible applications of DPSCs.

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Insects- Our Friends and Foe

Dr. N. Aarthi
Assistant Professor, Department of Zoology,
Sri Sarada College for Women (Autonomous),
Affiliated to Periyar University, Salem-16

Insects are small creatures found around us. Insects are the class which falls under the following systematic position

Kingdom : Invertebrata

Phylum : Arthropoda

Sub Phylum : Mandibulata

Class : Insecta

The class insecta is again classified based on the presence of wings into the following :

Sub class1: Pterygota (Winged Insects)

Sub class2: Apterygota (Wingless Insects)

Let us begin the chapter with a famous quotation of a great person **Edward O.Wilson**

"If all mankind were to disappear, the world would regenerate back to the rich state of equilibrium that existed ten thousand years ago. If insects were to vanish, the environment would collapse into chaos"

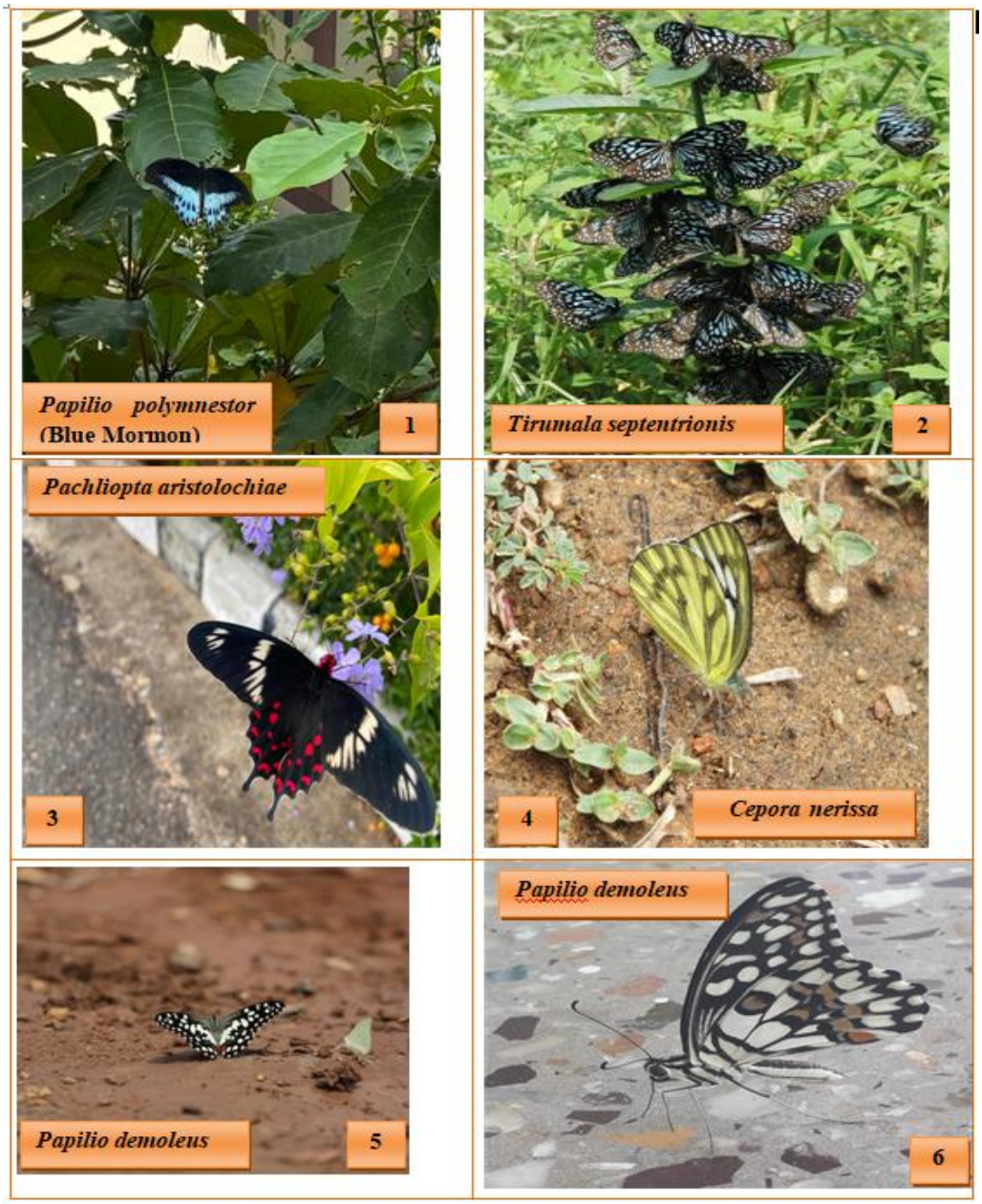
Insects' size varies from µ to Cm. The worlds of insects are very fabulous and amazing. We can see many insects around us. Study of insects is termed as "Entomology". Many insects play vital role in the environmental service and ecosystem. Let us see more information about a few insects in this chapter.

1. Dancing beauties of nature- The Butterflies:

Butterflies falls under the order Lepidoptera and it possess numerous species. Butterflies are very colourful and they add glitter and beauty to the environment. It recreates and refreshes mind. Apart from that Butterflies does environmental or ecosystem services like Pollinating plants, they are an indicator species Because butterflies are so sensitive to habitat and climate change, scientists are monitoring them as one way of observing the wider effects of habitat fragmentation and climate change. Therefore, an abundance of butterflies usually indicates a healthier ecosystem.

Butterflies maintain the ecosystem by acting as pollinator, prey, biological pest control, induce genetic variation in plants or heredity change which causes hybrid naturally and enhance environmental beauty, reduce the level of carbon dioxide in air. But butterfly population is decline rapidly and it is suggest that greater emphasis should be placed on management of habitat and better integration of protected areas. Ecologist use butterflies as model organisms to study the impact of climate change and habitat loss. The use of pesticides and other synthetic chemicals affects the innocent beauties. Puddling is a conspicuous behaviour in butterflies. Butterflies, mostly the male congregate and uptake the sodium and amino acids from the mud, dung, and urine of mammals or decaying flesh and then transferred to the female during mating. This behaviour is usually called as puddling. This also helps to clean the ecosystem.

Maharashtra has become the first State in the country to have a ‘State butterfly.’The BJP-led government has declared the Blue Mormon (*Papilio polymnestor*) as the State butterfly. The decision was taken at a meeting of the State Wildlife Board in Mumbai on Monday.


Papilio polymnestor
(Blue Mormon)
1
Tirumala septentrionis
2
Pachliopta aristolochiae
3
4
Cepora nerissa
Papilio demoleus
5
Papilio demoleus
6

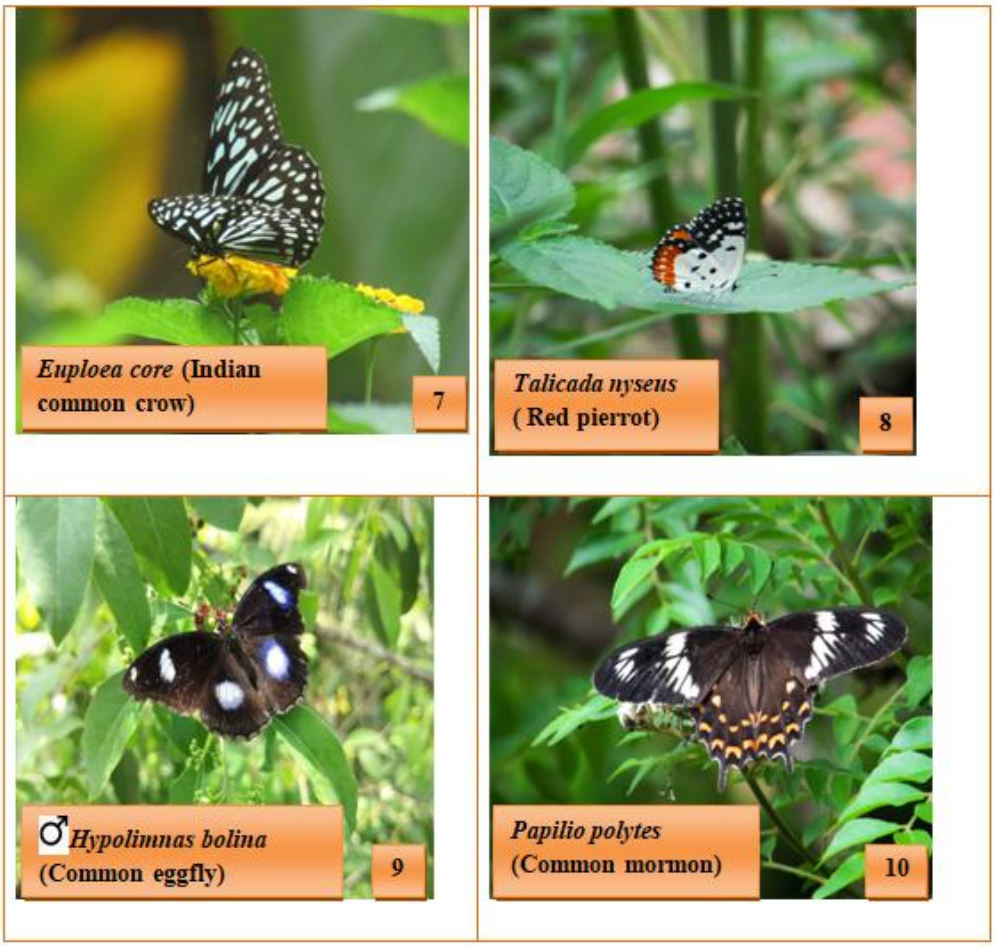
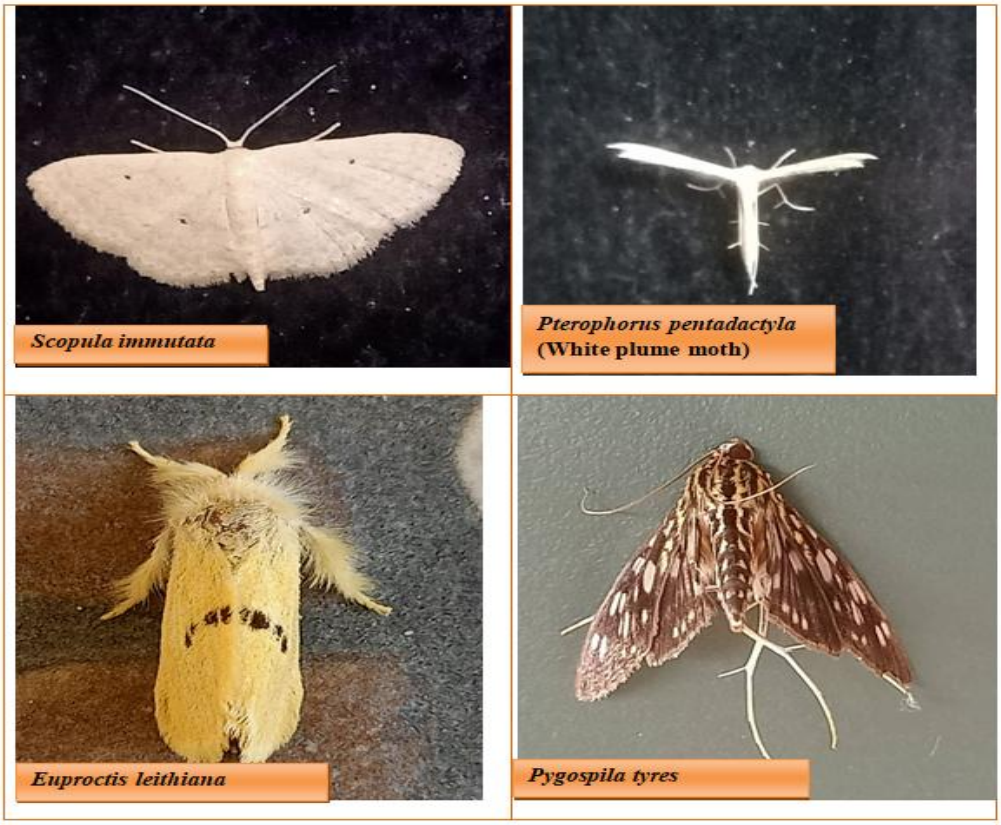


Image Courtesy 7, 8, 9, 10: Deeptha. T.C., II.M.Sc., Zoology, PSGR Krishnammal College for Women and Image 6: Dhanasubashini S, II.M.Sc., Zoology, PSGR Krishnammal College for Women, Coimbatore.

Next comes the moths which will be very similar to that of butterflies but they are nocturnal in nature as well as dark in colour to camouflage themselves. Moths belong to the order Lepidoptera. They have wide role in the ecosystem service. Though some moth caterpillars feed on the foliage the adults control the pests and to an extent help in pollination. Let us see about the interesting features of few moths around us. Since they are nocturnal and not brilliantly coloured many of us may not notice this pretty pitty moth. Moths are

sensitive to climate change. Use of pesticides and insecticides and habitat loss are major threats to its population.

Syntomoides imaon **image courtesy:** Deeptha.T.C., II.M.Sc., Zoology, PSGR Krishnammal College for Women.



The Oleander Hawk Moth, scientifically known as *Daphnis nerii*, is a fascinating creature that captivates the attention of entomologists and nature enthusiasts alike. With its notable features and intriguing behavior, it has become a subject of great interest. The adult Oleander Hawk Moth has a short lifespan, lasting only a few weeks. However, during this time, it plays a crucial role in the ecosystem. As a pollinator, it flits from flower to flower, transferring pollen and ensuring the continuation of plant species. Its graceful flight and gentle presence bring beauty and vitality to the natural world.

Scopula immutata (Geometridae), constituting one of the biggest families of Lepidoptera, are a species-rich and easily recognizable family that have served as indicators for environmental changes in many previous studies

Syntomoides imaon is a polyphagous pest that causes damage to various crops. *S*yntomoides *(Syntomidae-Lepidoptesa)* caterpillars feed on several agricultural and non-agricultural crop plants and affect the growth and yield of the crops. Some organisms are harmful to the humans also like *Syntomoides imaon*- handmaiden moth.

Next we can see about the **fascinating organisms in the universe of insects- Dragon flies**. Dragon flies belong to the order Odanata and it is a wonderful organism. The intricate venation of their transparent feathery wings is very beautiful and this plays a significant role in the environment. Odonata are considered to be good bio-indicators of environmental health and water quality because all the species within this order are dependent on water for the development of their pre-adult stages (commonly known as nymphs, naiads or larvae). The quality o the water or the water pollution due to heavy metals and other pollution are analyzed by monitoring the presence of dragon flies. Their ecosystem service is very crucial because many aquatic organisms are protected and saved by this insect by controlling the pollution.

Image courtesy ***Euproctis leithiana*****:** Deeptha.T.C., II.M.Sc., Zoology, PSGR Krishnammal College for Women and ***Diplacodes trivialis*****:** Dhanasubashini S, II.M.Sc., Zoology, PSGR Krishnammal College for Women, Coimbatore.

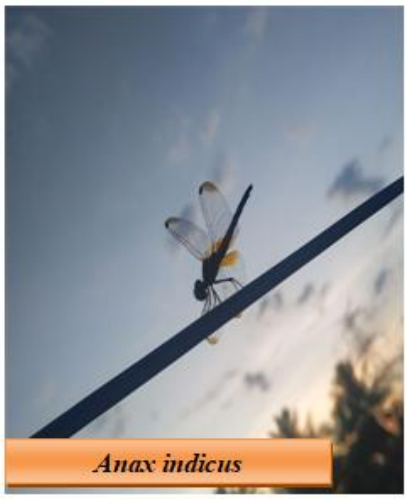


Image courtesy ***Pantala flavescens*****:** Deeptha.T.C., II.M.Sc., Zoology, PSGR Krishnammal College for Women and ***Anax indicus*****:** Roopika P, II.M.Sc., Zoology, PSGR Krishnammal College for Women, Coimbatore

Now comes the important insect- the cockroaches. Most of them feel irritating and disgusting if we say the name **cockroach**. They cause many infectious diseases to human and thereby it comes under the pests. Cockroaches fall under the order Blattodea.

Periplaneta americana spreads disease and thereby considered as foe**.** Many people show disgusting face when speak about cockroaches because of their habit and habitat. ***Therea petiveriana*** is another type of cockroach that is least concerned and need conservation too. They are typically omnivores, mostly feeding on decaying animal and plant matter. Some species feed on dead wood and are able to digest cellulose by intestinal symbiotic protozoans or bacteria. The cockroaches are generally found in litter among low vegetation or in the ground. Some species inhabit caves and few are associated with ant colonies. They are agile runners and they prefer to run than fly from potential predators. They tend to be nocturnally active - Spotted cockroach. They mimic or camouflage the six spot ***GROUND BEETLE*** (*Anthia sexguttata*) to save itself from predators. Hence this species of cockroach helps a lot in nutrient recycling process to make the soil fertile rich in humus.

Noisy Crickets: Crickets come under the order Orthoptera. They are nocturnal in habit and produces sound

Teleogryllus emma and *Teleogryllus commodus :* They are the species of Orthoptera and commonly called as emma field cricket. *Teleogryllus emma* presence indicates the species rich in the plant biodiversity. This *Teleogryllus emma* lives and feeds on variety of species. *Teleogryllus commodus* presence indicates the region as dry and rocky, since in between the cracks of the rocks it makes its habitat. They can be characterized by their wings folded on the sides of the body, Chewing mouthparts with thin antennae. *Teleogryllus emma* and *Teleogryllus commodus:* They are the species of Orthoptera and commonly called as emma field cricket. *Teleogryllus emma* presence indicates the species rich in the plant biodiversity. This *Teleogryllus emma* lives and feeds on variety of species. *Teleogryllus commodus* presence indicates the region as dry and rocky, since in between the cracks of the rocks it makes its habitat. They can be characterized by their wings folded on the sides of the body, Chewing mouth parts with thin antennae. Apart from the ecosystem service, both the species are cultured and reared artificially and reared for nutritional purpose.

Crickets serve rich protein for human being and it is now termed as entomophagy. Lolipops, candies infused with crickets, ice-creams and desserts with crickets are now getting popular some parts of the country to enrich the food with more proteins.

Next let us discover about **the wonderful carpenter organism- The Termites**

Termites comes under the order Isoptera and the scientific name is *Coptotermes formosanus*

When we hear the word ‘termite’, most of us are reminded of the irksome pests that eat away the wood in our houses, causing irreparable damage. However, did you know that termites also act as indicators of the health of an ecosystem, and their mounds. Termites are members of the Isoptera, an order with over 2600 described species in 281 genera. Two broad groups of termites may be distinguished on the basis of diet. Species that feed on humus, which are commonly found in tropical rain forests and build subterranean nests, depend entirely on partly decomposed plant matter in the soil. Wood- and litter-feeders, more abundant in savanna region. Termites contribute in the nutrient recycling by decomposing the solid wood and leaf litters to the soil. The presence of termitarium indicates the presence of more water in the soil. Presence of termitarium indicates the availability of water. This acts a bio-indicator to indicate the water. 579 termite mounds in the Bandipur Tiger Reserve, Karnataka, the team investigated the relationships

between the abundance and distribution of termite mounds with the soil properties and the fragmentation of natural forests. Termites are detritivores that eat dead and decaying organic matter like logs of wood in a forest. They form an important link in the cycling of nutrients in the forests by breaking down wood and organic residues on the ground, which would otherwise. Termites on the distribution of nutrients like carbon, nitrogen and phosphorus, at the ecosystemscale.

Let us figure out the web creature the Spiders:

Spiders falls under the class Arachnida and order Araneae. Approximately more than 40,000 species are identified and many are unknown. Let us figure out some of the species found around and help our environment to function without chaos. They are characterized by two body parts, the cephalothorax having 4 pairs of segmented legs and the abdomen. They have simple eyes, no antenna and no wings, which differentiate them from insects. Spiders are the key components of all ecosystems in which they live. Spiders were recognized as a generalist. Spiders are also well-known for their capacity to increase predation rates and also exhibited a functional response with respect to insect pest outbreaks. They are often predated on lepidopteran and

hemipteran pests which act as economically important pests of major crops or as vectors of plant diseases.

Plexippus paykulli species acts as a potential biocontrol agent against the *Musca domestica* and other small insects. The recent study revealed that *Telamonia dimidiata* also exhibits cannibalism, and, the hunter, in turn, became the hunted to another spider species. However, the study recorded an interesting observation while it was being eaten.

Phlegra fasciata also called as jumping spiders predates many small insects and helps in the maintenance of the foos chain and ecological energy flow. *Philodromus dispar* is also termed as running spiders. They won't build web and feed on small flies. They play an important role in regulating insect populations, serving as predators and helping to maintain balance in ecosystems. *Plexippus paykulli* preys are aphids, thrips, white flies, caterpillars, flies, and so on. It can be a very useful predatory organism to control insect pests. *Plexippus paykulli* is known from the literature as a polyphagous predator feeding on a wide variety of arthropod taxa including Odonata, Orthoptera, Homoptera, Lepidoptera, Diptera, Hymenoptera and other Araneae**.** *Pholcus phaangioides* commonly called as Daddy long legs have a relatively short lifespan, usually only living for a year or two. *Thomisus spectabilis* undergoes a unique colour transformation from yellow to white. The colour change helps them not only to hide from the predators but stalk prey similarly. They are important members of many ecosystems, as they help

to control populations of small insects and other arthropods.

Next come the tiny angels and active super stars always- The ants:

Ants are from the order Hymenoptera and it forms very big number of species. We can see different varieties of ants in and around us. They are very active and always live as colony with the pheromonal connection. The unity of ant is maintained with a chemical messenger called pheromones. They don't have ears but sense with the earth's vibration. Provisioning services are goods or services provided by organisms that directly improve human well-being; examples include the provisioning of food, timber, and fiber .Here we describe two ways in which ants provide a product or service which directly promotes

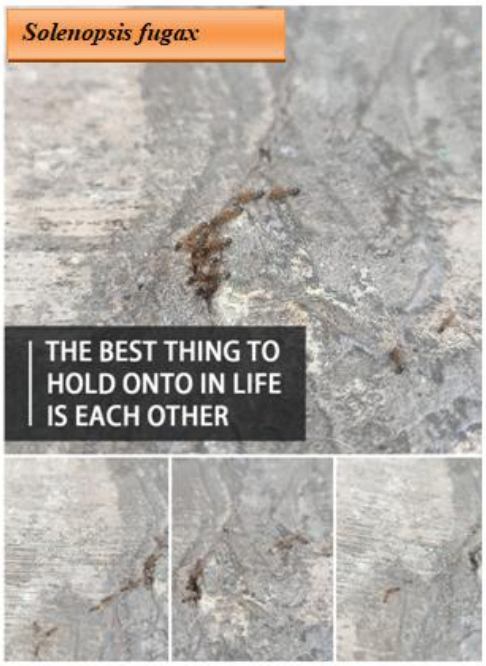
human well-being by providing material goods, and sustaining health and security: (I) the use of ants as food resources, and (II) the use of ants in medical and pharmaceutical applications. Entomophagy, or the use of insects as food, is a provisioning ecosystem service frequently. Ants are also providers of biomedical services arising from biotechnological developments and pharmaceutical products. Recent developments of treatments for the potentially deadly anaphylactic reactions that sometimes result from ant stings ironically are derived from the ant venom itself, a treatment known as immunotherapy, in which the patient's immune response is enhanced by small dosage exposure to the ant venom. This has been particularly well explored and experimentally tested with *Solenopsis invicta*

The Weaver ant is wonderful creature and there are many things to wonder the nature. The adult will not build the nest. The larva of weaver ant *Oecophylla smaragdina* secretes a silk like substance and the adult holds the larva in its hand and stitches the leaves together to form the nest. Ants play a vital role in recycling nutrients, Pollinators, dispersing seeds and plant parts, engaging in mutualistic associations with other fungi species, and serving as predators and scavengers. The ant species of Crematogaster and Oecophylla were dominant on the tree trunk which nested on trees.

Many scientist works on ants to render information about their ecosystem services to the public people and henceforth to conserve the ants. One of the study reported that the presence of Ants correlated with higher yields across five crop types, with average increases of 7.96% in cacao (*Theobroma cacao*), 55.75% in cashew (*Anacardium occidentale*), 16.47% in citrus, 49.51% in coconut palm (*Cocos nucifera*), and 30.37% in mango (*Mangifera indica*) orchards. Ants reduced pest density and/or damage for herbivores in seven insect families (Chrysomelidae, Coreidae, Curculionidae, Miridae, Pentatomidae, Tephritidae, and Thripidae).

Ants and aphids have a special symbiotic relationship; ants will collect aphids in your garden before they can destroy the plants in order to obtain food from the aphids. In return, the aphids are provided security by the ants against predators. This culling of aphids is called "**farming**" or "**aphid-herding**." Aphids carry a sweet, honey-dew type liquid in their bodies that ants love. Ants love it so much that they actually keep a "corral" of aphids to have the sweet honey liquid whenever they desire it. When an ant is hungry, she taps the aphid with her antenna or gently strokes the aphid to let it know she would like some honeydew. The aphid then excretes the honeydew to feed the ant. Ant "farmers" are able to keep a consistent supply of honeydew on hand for

the enjoyment. **Ants can be beneficial** to the environment in a variety of ways. Ant colonies aerate and enrich the soil, creating a stable ecosystem by recycling dead animals, insects, and decaying matter, placing nutrients back into the soil. Ant tunnels enhance water infiltration and circulate air in the ground which is beneficial not only to the soil, but plant roots as well. Ants also help with pollination by crawling over one flower to another; in the home and outside, ants are natural pest control agents for termites (ants eat them). Certain species such as **Seed-harvesting ants** collect and unwittingly spread seeds, providing a new harvest of plants. *Myopopone castanea* is a common ant species found in India, which nest under barks of trees or dry logs. This is the only extant species of the genus and is considered a living fossil. The large black ant (Camponotus compressus), the Indian army ants which form long trails (*Leptogenys chinensis*), the trap jaw ants (*Odontomachus monticola*) etc are common in the western Ghats." When quizzed about the role of ants in the ecosystem, pat comes the reply, "Ants play an important role in the ecosystem as predators, seed dispersers, scavengers and pollinators. They are the most important predators in the ecosystem and hence are natural pest controllers. As scavengers, they remove carcasses and other organic materials. The granivorous ants are important seed dispersers."



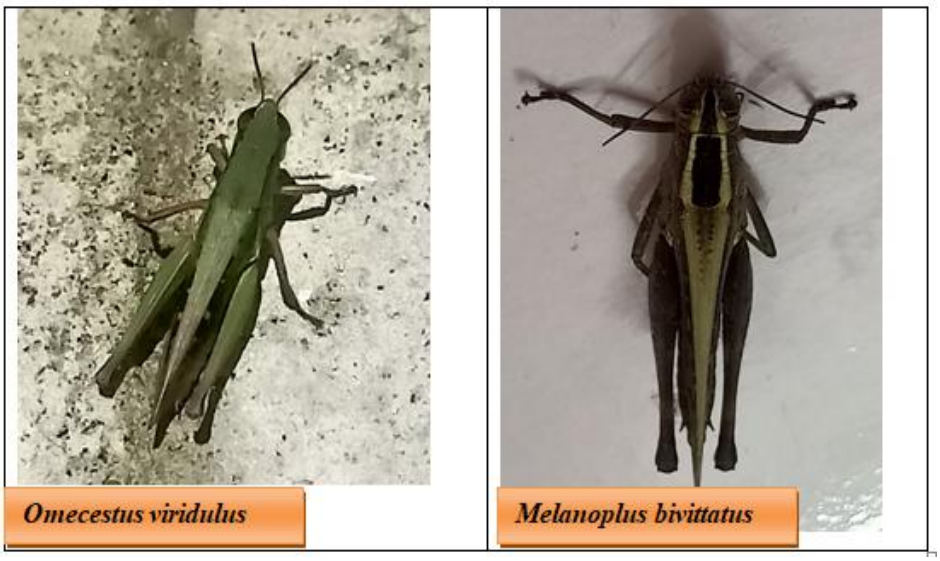

Weaver ant- Oecophylla longinoda


Weaver ant- Oecophylla longinoda


Solenopsis invicta

Next serves the nature by its active part in food chain and energy flow- the Grasshoppers.

They come under the order orthoptera. *Melanoplus bivittatus* is a yellow striped grasshopper. The species hs yellow green colour all over the body is due to the presence of chromoprotein and carotenoid. They provide protein rich food to their predators and they help in cutting the foliages which helps in fast recycling through the decomposers, which would otherwise take longer duration for the recycle of leaves and litters. Hence the main environmental service and role in ecosystem is to help in the litter management. *Omecestus viridulus* also helps in the same nutrient recycling.



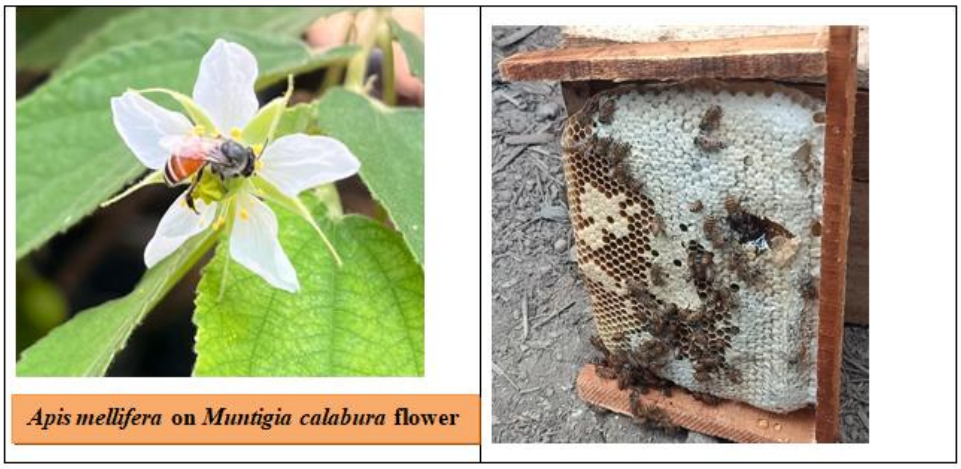
Battle the predators with the beetle: Beetles belong to the order Coleoptera. The *Aulacophora* species are great pest to the pumpkin plant. They cause severe damage to the plants. They attack the leaves and will not allow the plants to flower properly and if at all lowers they shed off and the fruit yield will be totally reduced and caused loss to the farmers. *Batocera rufomaculata* is a beetle which bores the stem or trunk of the *Manifera indica* which leads to hectic economic fall to the farmers. Hycleus pustulatus is called as blistter beetle and they are polyphagous insects which feeds on the flowers and also helps in pollination unknowingly. *Oryctes rhinoceros* beetle cause damage to coconut trees and this affects the entire tree and very dangerous pest to *Cocos nucifera.* The darkling beetle *Blapstinus fortis* plays a major role in soil fertility by nutrient recycling. They decompost the dead leaves and plant debris to rich humus.

Aulacophora Sps

Succeeding is our eusocial and very essential organism in the insects, the sweet little honey bees. Honey bees come under the roof or order hymenoptera. There are different types of bees and they play very important ecological service like pollination. When we talk about pollination, I remember a quote by famous scientist Albert Einstein that

"If the bees disappeared off the surface
Of the globe then man would only have
Four years of life left "

This is because if no more bees then no pollination and no plants and no animals and no life on Earth. Honey bees serves the environment by pollinating, serves the human society with many products like honey, royal jelly, bee bread, bee wax, pollen and propolis. All these products are very healthy and nutritious which serves as supplementary diet. All are aware of honey bees and many entrepreneurs are developing nowadays in the ield of Apiculture- Rearing of honeybees.



Apis mellifera on *Muntigia calabura* flower

Conclusion:

Insects play many roles in the ecosystem. Some insects are beneficial and some are harmful. Some insects like mosquitoes and fleas spread many deadly diseases as a vector. Human interruption in the insect world is very enormous and causes high threat to many economically, ecologically important insects like butterflies, honey bees, dragon flies etc. Arthropods have long been recognized as important in the functioning of soil ecosystems, and a vast

literature accordingly has accumulated. Much of the earlier literature, still relevant today, was cited in several classic treatises on the biology of soils published beginning in the 1950s. These insects provide aesthetic and ecstasy to kids and adults. Many insects secretions are used in the pharmacology as drug and used as remedial treatment. Such wonders of nature need to be conserved. Many toxic pesticides not only targets the pests but also other innocent creatures are also trapped. Stop using synthetic pesticides and can amend with bio-control (using predatory insects to kill the pests). Create awareness among friends and neighbours that Insects as our friends and foe. Even foe can be treated in a legible manner which does not affect the other friends. Conserve nation's natural treasure- the insects by rejoicing it and not by regretting.

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An overview of the herbs used to treat conjunctivitis

[1]Ajay Kumar Shukla, [1]Vimal Kumar Yadav, [1]Vishnu Prasad Yadav, [1]Aarti Tiwari, [2]Pallavi Tiwari, [3]Garima Verma, [4]Manoj Kumar Mishra, [5]Maya Sharma

[1.] Institute of Pharmacy, Dr Ram Manohar Lohia Avadh University Ayodhya U.P, India.

[2.] Basic Education department, Sultanpur, U.P, India

[3.] Department of Pharmaceutics, Swami Vivekanand Subharti University, Meerut, U.P.

[4.] Department of Pharmacy, Shambhunath Institute of Pharmacy, Prayagraj, U.P, India

[5.] Faculty of Pharmacy, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan,India

*Corresponding Author E-mail: *ashukla1007@gmail.com*

Abstract

An overview of the herbs used to treat conjunctivitis is provided in this review article. It is frequently called "pinkeye." Conjunctivitis outbreaks have occurred all over India, encouraging the usage of local herbal remedies for ages. Conjunctivitis is uncomfortable and, despite typically not being a serious condition, can occasionally result in corneal involvement, which can cause partial or total blindness. Eye redness and swelling, irritation and increased tearing, and photophobia are all symptoms. Treatment options for chronic noninfectious conjunctivitis include antihistamines and sodium cromoglycate. Extremely contagious bacterial, fungal, or viral infections have sparked epidemics in Asia, Africa, and India. *Staphylococcus aureus, Chlamidia trachomatis*, adenoviruses, Enterovirus 770 (EV 70), and Coxsackie virus are examples of common pathogens. This study demonstrates the wide range of herbal remedies for conjunctivitis, including licorice, honey, fennel, red clover, pokeroot, turmeric, marigold, and other.

Keywords: Conjunctivitis Skin, herbal remedies, uncomfortable, turmeric, marigold

Introduction

Infection or inflammation of the clear membrane lining your eyelid and eyeball causes pink eye. Itching and redness in the eyes are typical symptoms, as well as a gritty sensation. On occasion, a discharge will crust over on your eyelashes at night. The preservation of a healthy cornea and subsequently the eye's visual acuity depend on a healthy conjunctiva. The conjunctiva contributes to the three-layered tear film.[1]

Conjunctival infections, such as gonococcal infections, can travel to the cornea and result in a perforation. The cornea may become affected by limbal catarrh or allergic conjunctivitis. The tarsal conjunctiva under the lid develops "cobblestones" that can lead to corneal ulcers. The limbal stem cells that are responsible for the cornea's re-epithelialization can be eliminated by chemical harm to the area. The cornea's surface will become damaged due to dryness.

Signs and Symptoms [2]

The following signs of conjunctivitis can appear in one or both eyes:

Tearing and redness Itching Swollen eyelid

Discharge (thick or watery)

Overnight crust, sensitivity to light, and gritty sensation.

Physiology of the Conjunctiva :[3]

The conjunctiva is a thin, transparent mucous membrane that covers the front surface of the eye and lines the inside of the eyelids. It plays several important roles in maintaining eye health and function. Here are some key aspects of the physiology of the conjunctiva:

Protection: The conjunctiva acts as a protective barrier for the eye, helping to shield it from foreign particles, microbes, and environmental irritants. It produces a thin layer of mucus that helps trap and flush away debris and pathogens.

Lubrication: The conjunctiva contains goblet cells that secrete mucus, contributing to the tear film that covers the surface of the eye. This mucus layer helps to keep the eye moist and lubricated, preventing dryness and discomfort.

Nutrient Supply: Blood vessels in the conjunctiva provide a rich supply of oxygen and nutrients to the outer layers of the eye, including the cornea. This vascular network ensures that the eye receives the nourishment it needs for proper function.

Immune Defense: The conjunctiva contains immune cells, such as lymphocytes and macrophages, which help protect the eye from infections. These cells are part of the body's immune system and play a role in recognizing and eliminating pathogens that may come into contact with the eye's surface.

Sensation: The conjunctiva contains numerous nerve endings that contribute to the eye's sensitivity to touch, foreign objects, and irritants. This sensitivity helps trigger protective reflexes, such as blinking, to remove potential threats from the eye's surface.

Tear Production: While the main source of tears is the lacrimal gland located above the eye, the conjunctiva also contains accessory tear glands known as accessory lacrimal glands. These glands produce a portion of the tear film, helping to maintain the eye's moisture and protect its surface.

Transparency: The conjunctiva is transparent, allowing light to pass through it and reach the cornea and lens of the eye. This transparency is essential for clear vision.

Conjunctivitis (Pink Eye) in Babies & Children :[4]

Pink eye, often known as conjunctivitis, can affect infants and children just like it can adults. The thin, transparent membrane that lines the inside of the eyelids and covers the white component of the eye is called the conjunctiva, and pink eye is an infection of it. Viruses, germs, allergies, or irritants are just a few of the possible causes. What you should know about conjunctivitis in children and babies is provided below:

Symptoms: The main symptom of pink eye is redness and swelling of the whites of the eyes. Other common symptoms can include:

Excessive tearing

Itchiness or discomfort

Sensitivity to light

Discharge from the eye that may be clear, white, yellow, or green, depending on the cause

Types of Conjunctivitis:

Viral Conjunctivitis: Often caused by a virus like adenovirus, and it can be highly contagious. It tends to be accompanied by watery discharge and is often associated with a recent cold.

Bacterial Conjunctivitis: a disease brought on by bacteria like Staphylococcus or Streptococcus. The discharge is thicker, yellow or green.

Allergic Conjunctivitis: Occurs as a result of exposure to allergens like pollen or pet dander. It often involves both eyes and is associated with itching and clear discharge.

Irritant Conjunctivitis: Can result from exposure to irritants like smoke, chlorine, or foreign objects in the eye (5-6).

Treatment:

Typically, viral conjunctivitis goes away on its own and doesn't need any special care. Utilising cool compresses and keeping the eye clean can also help ease discomfort. A doctor's prescription for antibiotic eye drops or ointment is typically used to treat bacterial conjunctivitis. Avoiding allergens and taking antihistamine eye drops or oral drugs as directed by a healthcare provider are two ways to treat allergic conjunctivitis.

Irritant conjunctivitis can be alleviated by flushing the eye with clean, cool water and removing the source of irritation

Prevention:

Practicing good hygiene, including frequent handwashing, can help prevent the spread of infectious conjunctivitis.

Avoiding close contact with infected individuals can reduce the risk of transmission.

Ensure that children with pink eye don't share towels, pillowcases, or other personal items.

If your child has allergic conjunctivitis, identifying and minimizing exposure to allergens can help prevent recurrence.

When to See a Doctor:

If your child has severe eye pain, vision changes, or persistent symptoms, consult a healthcare professional promptly.

If your baby (especially newborns) shows signs of conjunctivitis, it's essential to seek immediate medical attention, as it could be a more serious condition.

Remember that it's crucial to consult a healthcare provider for a proper diagnosis and treatment plan if you suspect your child has conjunctivitis. They

can determine the cause of the infection and recommend the appropriate treatment based on the specific type of pink eye (7-10).

Supplements and herbs :[11-13]

Conjunctivitis, also referred to as pink eye, is an inflammation of the conjunctiva, a thin, transparent layer of tissue that lines the inside of the eyelids and covers the white of the eye. Even while it usually resolves on its own or with medical care, there are several nutrients and herbs that may offer relief or aid in the healing process. However, since supplements and herbs can conflict with prescriptions or underlying medical issues, it's imperative to speak with a healthcare provider before utilising any. Here are some recommended vitamins and herbs for conjunctivitis:

Vitamin C: Vitamin C is an antioxidant that can support the immune system and may help speed up recovery from infections like conjunctivitis.

Vitamin A: Vitamin A plays a vital role in eye health. However, excessive vitamin A intake can be harmful, so it's essential to consult a healthcare provider before using vitamin A supplements.

Zinc: Zinc is known for its immune-boosting properties and may help in the healing process.

Omega-3 Fatty Acids: These essential fatty acids are beneficial for overall eye health. They can be found in fish oil supplements.

Eyebright (Euphrasia): Eyebright is an herb that has been traditionally used to treat eye conditions, including conjunctivitis. It may have anti-inflammatory and soothing properties.

Chamomile: Chamomile is another herb known for its anti-inflammatory and calming effects. It can be used as a warm compress or in an eyewash to soothe irritated eyes.

Goldenseal: Goldenseal contains berberine, which has antimicrobial properties. It may help fight off bacterial or viral conjunctivitis. However, it should be used with caution and under the guidance of a healthcare professional.

Aloe Vera: Aloe vera gel can be applied topically to soothe irritated eyes and reduce inflammation.

Calendula: Calendula is another herb that may have anti-inflammatory and antimicrobial properties. It can be used in eyewashes or compresses.

Turmeric: Curcumin, the active compound in turmeric, has anti-inflammatory and antioxidant properties. It may help reduce inflammation and soothe the eyes.

When using supplements or herbs for conjunctivitis, it's crucial to follow these guidelines:

Consult a healthcare provider: Seek professional advice before using any supplements or herbs, especially if you have underlying health conditions or are taking medications.

Topical application: For herbs like chamomile, calendula, and aloe vera, use them topically as a warm compress or eyewash. Ensure that the preparations are sterile to avoid introducing new infections.

Dosage and safety: Follow recommended dosages and safety guidelines for any supplements or herbs you choose to use.

Hygiene: Maintain proper eye hygiene, including washing your hands and avoiding touching or rubbing your eyes.

Remember that while these supplements and herbs may offer some relief or support, they should not replace conventional medical treatment for conjunctivitis. If your symptoms persist or worsen, consult a healthcare professional for appropriate diagnosis and treatment.

Herbs :[15-17]

The use of herbs is a tried-and-true strategy for strengthening the body and curing disease. However, herbs can interact negatively with other herbs, nutritional supplements, and medications. Use herbs with caution and only under the direction of a doctor who has obtained training in botanical medicine. Eye washes and compresses are examples of external therapy. The use of herbal eyewash may be advised by a trained herbalist. Here are some instances when herbs have been used in these treatments.

Euphrasia officinalis, often known as eyebright, helps dry up excess fluid and fights infection.

Chamomile (Matricaria recutita) aids in the prevention of infections.

Fennel seed, or Foeniculum vulgare, aids in the battle against infection.

Calendula officinalis, sometimes known as marigold, calms inflammation.

Plantain, or Plantago lanceolata, has astringent and calming properties. The most potent plant portion is the young leaves.

Other natural remedies could include ginkgo biloba extract combined with hyaluronic acid. In one study, compared to using hyaluronic acid alone, utilising eyewash consisting of this solution for a month significantly reduced conjunctivitis symptoms. Many health food stores sell pre-made herbal eyewashes. Many of them contain goldenseal (Hydrastis Canadensis) diluted solutions, which can be exceedingly irritating to the eyes in undiluted forms. Observe the manufacturer's instructions closely [15-17]

Preparation of home remedies and list of herbs :[18-21]

Liquorice root

The licorice plant's root extract might have medicinal benefits for treating keratitis and conjunctivitis symptoms. Anti-inflammatory properties of licorice may help to lessen swelling and related irritation on the surface of your eyes. Some people think licorice can treat viral or bacterial diseases as well.

The elderberry

The fruit of the elder tree is used to make elderberry extract. Inflammation-reducing effects exist in this extract. Theoretically, this might help reduce inflammation brought on by keratitis and conjunctivitis. Elderberry may also be beneficial for viral infections. Your doctor will go over how to use elderberry and when you should stop taking the extract if you and your doctor decide that it could assist with your symptoms.

The Ginger

The versatility of ginger as a flavouring and seasoning ingredient in food is widely known. Additionally, this supplement might be medical in nature and assist reduce inflammation. A ginger supplement may be advised by your doctor if you have viral conjunctivitis or keratitis, both of which are viral infections that cause symptoms of the common cold. Your doctor can advise you on the appropriate form of ginger to use for your ailment. Ginger comes in powered, dry, or whole root form. Consult your doctor before beginning a treatment with garlic. Since ginger may interfere with blood-thinning medication, it may not be the ideal treatment for your eyes, especially if you take anticoagulant medication.

Hot salt water

Put some water in a coffee mug. After filling it halfway, I microwaved it for 4 minutes. 2 1/2 tablespoons of salt were added. I waited until the hot salt water had cooled, then took a piece of paper, and for 20 minutes, I just squeezed the warm salt water into my eyes five times every five minutes.Continue till you look better.

Ice-cold milk

Pour some milk from a cup of cold milk into your eye, then repeat the process until you feel better.

I poured a dab of coconut oil (all-natural from Whole Foods) over my eye and allowed it to absorb. The majority of the redness was instantly reduced, making this the most effective remedy. Apply before bed because vision was fuzzy for around 30 minutes following.)

Golden Seal (simply some boiling water with some Golden Seal powder added, and a dab on the eye). Eventually, when the water has fallen directly into your eye, add a drop using a straw.)

"Aloe vera."

Rose water (a combination of 2, 3, and 4).

Seawater is water with sea salt.

Comfrey and distilled water solution.

Distilled water and the golden seal solution.

Distilled water with apple cider vinegar.

A solution of boric acid.

Other beneficial herbs :[22- 24]

Celery, Fumitory, Licorice, Meadowsweet, Motherwort, Pokeroot, Raspberry, Red Clover, Self-Heal, Turmeric, Walnut, and Walnut etc

Drug Therapies :[25-28]

A doctor may typically recommend antiviral eye drops, such as acyclovir trifluridine, for viral conjunctivitis. Supportive care may entail the use of artificial tears and ocular decongestants. Applying cold compresses three times per day for one to three weeks may ease discomfort.

Conjunctivitis allergic: To lower your sensitivity to the allergen, your doctor may recommend allergy shots given over several months. Eye drops containing antihistamines, such as those offered over-the-counter under the name Vasocon-A or on prescription under the name Patanol, may aid in reducing swelling or discomfort. Antihistamines used orally can help with itching reduction. In order to relieve symptoms, a cold compress may be helpful. For bacterial conjunctivitis, doctors frequently prescribe eye drops with antibiotics like sodium sulfacetamide or azithromycin or ointments with antibiotics like erythomycin, bacitracin, or neomycin.

Corresponding and Alternative Therapies :[29-31]

Alternative forms of treatment can ease discomfort. Any liquid or compress applied to the eye must, however, be sterile.

It's important to remember that conjunctivitis can move from one eye to the other. As a result, keep the opposite eye away from the treated one.

Start with compresses if you only have a minor case of conjunctivitis. To treat allergic or irritative conjunctivitis, apply cold compresses as opposed to warm ones. Use an eyewash and pre-mixed compress for a small case; alternatively, buy them from a certified herbalist.

Homeopathy Treatments :[32-34]

A severe form of allergic conjunctivitis, marked by a stringy discharge, swollen eyelids, scaly skin, and excruciating agony, can occasionally appear in sufferers. To avoid corneal scarring, this condition requires aggressive treatment.

Name of plants	Uses
Euphrasia	Conjunctivitis with watery tears that burn the face and can develop into a thick discharge is treated with euphrasia. Even though watery tears are being produced, the person may still have a gritty, dry feeling in their eyes
Argentum nitricum	*Argentum nitricum* for red, swollen eyes with pus-like discharge and splintering pains.
Pulsatilla	Pulsatilla for conjunctivitis that may come up during or right after a cold and cause itchy eyes and a yellow-green discharge. Cold compresses typically alleviate symptoms, which include the tendency for the eyelids to stay together. Most people who are prone to irritability and mood swings should use this therapy

Belladonna	Belladonna is used to treat conjunctivitis in its early stages, which are characterised by sudden onset of burning, bloodshot eyes, swollen eyelids, and light sensitivity. Typically, the touch of the eyes feels heated and aching
Sulphur	Sulphur is used to treat eyes that are burning, hurting, and red and have a yellow discharge that smells bad. The person is usually quite hot and thirsty, and the eyes are frequently crusted together

Prevention of infective conjunctivitis :[35-38]

Bacterial conjunctivitis can be spread through touch with diseased hands or upper respiratory tracts, though this is uncommon. Gonorrhoea can be spread from the vaginal area or urine by contacting the eye. There has been a serious breach of hygiene here. To prevent ophthalmia neonatorum, infants can be treated with antibiotics, povidone iodine drops, tetracycline eye ointment, and other antiseptics or antibiotics. Viral conjunctivitis caused by an adenovirus can quickly spread throughout a neighbourhood or a building like a school. Towels, face cloths, hands, and applanation tonometers are just a few examples of how this can easily spread and needs to be managed by the application of strict hygiene standards. In order to prevent allergic conjunctivitis, a patient must be able to modify their surroundings or occupation, or they must be able to recognise and avoid the allergen that is causing their allergies, such as pollen or animal fur. Drugs may create allergies, which can be treated by ceasing the drug. Such medication responses are frequently caused by atropine, neomycin, and eye drop preservatives.

Conclusion

There is a tonne of literature on conventional usage of many herbs for conjunctivitis, but there has been very little systematic research on the clinical applications of the plants, as is the case with many herbal medications. According to the authors, many traditional herbal eye medicines are probably safe, and some of them might even be helpful. To assure their safety and lower the likelihood of harmful side effects or difficulties, they emphasise the necessity of standardisation as well as the manufacture of pure herbal ophthalmic medications. Any researcher interested in researching the use of herbs in the treatment of conjunctivitis can use this thorough review as a jumping off point. Finally, mention how conjunctivitis caused by bacteria and viruses is both extremely contagious. Use different towels for each member of

the family. Wash your hands regularly. Children should not attend creche or school. When taking any drug, especially antibiotics or corticosteroids, always follow your doctor's instructions. Keep your contact lenses clean if you wear them to prevent recurring infections and irritation. Wear them once your eyes have healed, then stop. A severe form of allergic conjunctivitis, marked by a stringy discharge, swollen eyelids, scaly skin, and excruciating agony, can occasionally appear in sufferers. To avoid corneal scarring, this condition requires aggressive treatment.

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Vitamin D and Periodontal disease

Aruna. K.M[1*] and Deepa Rajendiran[2]
[1*]General Practitioner, Oral and Maxillofacial Pathologist,
Om Sakthi homes, thiruvalluvar second street, Adambakkam, Chennai
[2] Associate Professor, Department of Biochemistry,
Madha Dental College and Hospital, Chennai, Tamil Nadu, India.
E-mail: *deepanish1981@gmail.com*

Abstract

Periodontal disease is one of the most prevalent oral diseases with multifactorial etiology. The etiology ranges from microbial causes to systemic diseases which also includes nutritional deficiencies. Vitamin D is one such nutrient that plays an important role in the regulation of periodontal health. Deficiency of vitamin D results in alteration of periodontal status leading to diseased condition. This is mainly attributed to the effects of vitamin D on bone metabolism and the regulation of immune responses. Vitamin D is also thought to have antibiotic effects against periodontopathogens which alters the disease progression. Vitamin D supplements are thought to improve periodontal health. This article aims to review the role of vitamin D in periodontal disease and health.

Keywords: vitamin D, periodontal disease, bone remodeling, immunomodulator

Introduction

Vitamin D is a nutrient long considered as essential for skeletal health but is now attracting interest from medical communities as knowledge emerges of its various biological functions and its association with the decreased risk of many chronic diseases.[1]

Periodontal disease is widely accepted as a chronic host-mediated reaction that causes periodontal destruction by releasing pro-inflammatory cytokines by local tissues and immune cells in response to the bacteria of the dental plague and their products and metabolites. Vitamin D through its effect on bone and mineral metabolism, immune response, and through Vitamin D receptor, is now believed to benefit periodontal health and its deficiency is associated with periodontal disease.[2]

Hence, vitamin D must be brought into the spotlight to emphasize its role in the maintenance of periodontal health. This article aims to review the role of vitamin D in periodontium and the association of vitamin D with periodontal disease progression.

Vitamin D

Vitamin D is often referred to as the 'sunshine vitamin' since it can be synthesized by our skin on exposure to sunlight. Vitamin D known as calciferol is available in two forms vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Vitamin D2 is mainly obtained from plant sources. Vitamin D3 is produced by our skin on exposed to sunlight.[3] Vitamin D3 synthesized by our body is inert and undergoes a physiological process to change into active form 1,25-Dihydroxyvitamin D3[4].

Vitamin D synthesis

7- dehydrocholesterol in the skin on irradiation by ultraviolet light of 290- 315 nm gets converted into vitamin D3 (inactive form). Vitamin D3 undergoes first hydroxylation in the liver by the enzyme 25-hydroxylase to form 25-hydroxyvitamin D3 which undergoes further hydroxylation in the kidneys by the enzyme 1-hydroxylase to form 1,25-Dihydroxyvitamin D3 which is the active form. 1,25-Dihydroxyvitamin D3 is considered as a steroid hormone (Decostriol) and this is because it was thought to act like other steroid hormones and also interacts with VDR (vitamin D receptor).[4] Serum levels of 25-hydroxyvitamin D 10≤ng/dl indicates severe deficiency, levels of 10-20 ng/ml indicates deficiency and between 21-29 ng/ml suggests insufficiency of vitamin D[5].

Actions of vitamin D

Vitamin D plays a vital role in maintaining homeostasis in our body. vitamin D has skeletal as well as extra-skeletal effects. The extra skeletal effects are seen on the intestine, kidneys, cancer cells, immunity, muscles, cardiovascular system, and diabetes[1]. The biological actions of vitamin D are depicted in Figure 1.

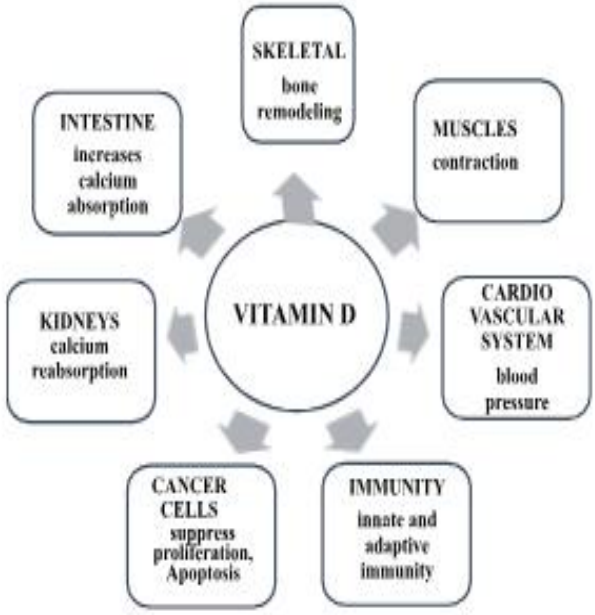

Figure 1: The biological actions of vitamin D

Vitamin D and periodontal disease

Studies have proven an interesting association between vitamin D and periodontal disease. In a study conducted by Patil et al.[6], 48 chronic periodontitis and 37 periodontally healthy controls were included. The study concluded that 97.9% of chronic periodontitis patients demonstrated 25(OH)D3 deficiency or insufficiency, whereas only 2.1% of the participants had sufficient levels of 25(OH)D3. Studies by Boggess et al.[7], Chang et al.[8], Antonoglou et al. [9] and Abreu et al. [10] also have shown an association between low Vitamin D3 levels and periodontitis.

Vitamin D plays an important role in maintaining bone mineral density which is a contributing factor for periodontitis characterized by bone loss and mobility of teeth.[2] Serum levels of vitamin D were significantly and inversely associated with periodontal attachment loss independent of bone mineral density.[5] The effects of vitamin D in periodontitis are mainly attributed to the inhibition/ suppression of adaptive immunity through inhibition of inflammatory cytokines and stimulation of innate immunity through monocytes/macrophages to secrete molecules with potent antibiotic effects.

These anti-inflammatory and antibiotic effects play an important role in disease suppression.[2]

It is also true in the case of gingivitis where deficiency of vitamin D is correlated with gingival inflammation. A linear association between Vitamin D and bleeding on probing exists. Vitamin D supplements can be used as anti-inflammatory agents to treat gingivitis.[11] Studies have proven that deficiency of vitamin D leads to periodontal disease progression due to its role in bone remodeling and also due to its immunomodulatory and anti-inflammatory properties.

Vitamin D and bone metabolism

It is well-established fact that vitamin D plays an important role in calcium homeostasis. Vitamin D aids in the absorption of calcium from the small intestine and also from the bone by resorption. In the osteoblasts, Vitamin D interacts with the receptor (VDR) and increases the expression of RANKL (Receptor Activator for Nuclear Factor κ B Ligand). Whereas, RANK present on the preosteoclasts interacts with RANKL on the osteoblasts. This leads to the conversion of preosteoclasts into osteoclasts which causes bone resorption. The acidic factors like hydrochloric acid released during this conversion metabolize calcium from the bones and allow it into the circulation. Thereby calcium homeostasis is achieved in the blood.[12]

The biologically active form of vitamin D helps in maintaining calcium and bone homeostasis which results in increased mineral density of alveolar bone and thus may reduce alveolar bone resorption, with a consequent decrease in the severity of chronic periodontitis and may help to maintain periodontal health.[13]

Vitamin D as immunomodulator

Vitamin D acts as an immunomodulating agent; this is mainly attributed to the presence of vitamin D receptor (VDR) in the immune cells like B cells, T cells, and Antigen-presenting cells. Vitamin D influences both innate and adaptive immunity. Deficiency of vitamin D will result in increased susceptibility to infection.[14]

Vitamin D is produced by the keratinocytes of the basal and spinous layers of the oral epithelium and it influences proliferation, di erentiation, and apoptosis of keratinocytes, and local immune responses.[15]

Vitamin D and innate immunity

Vitamin D enhances innate immunity by enhancing the chemotaxis and phagocytic capabilities of innate immune cells like monocytes and macrophages of the periodontium which activates the antimicrobial peptides like defensin B and cathelicidin which are capable of destroying the microbe's cell membrane.[16]

Dendritic cells (DC) are an important component of the immune system that forms a link between innate and adaptive immunity. They have two major phases in their life cycle. Immature Dendritic cells capture the antigens and process them initiating a primary immune response. On maturation, DCs migrate towards the regional lymph nodes and become antigen-presenting cells that present the processed antigen to the antigen-specific lymphocytes thereby initiating the adaptive immune response. Vitamin D suppresses the differentiation and maturation of dendritic cells. As the maturation of dendritic cells is inhibited by vitamin D, it impairs the stimulatory capacity of antigen-specific lymphocytes. [17,18]

Vitamin D and adaptive immunity

Vitamin D suppresses the adaptive immune response. It affects both B-cells and T-cells. It suppresses the immune responses mediated by type 1 helper (Th 1) cells. This in turn results in the suppression of inflammatory cytokines like interleukin-2 (IL-2), interferon-gamma (INF γ) produced by the Th 1 cells.[19] vitamin D causes inhibition of memory and plasma cell generation by the B cells and promotes apoptosis of immunoglobulin-producing B cells.[20]

Vitamin D and anti-inflammatory properties

Vitamin D induces the regulatory T (T_{reg}) cells that is responsible for the inhibition of inflammation.[19]

With all this evidence, it is known that vitamin D has an important role in the immune system both innate and adaptive immunity. Deficiency of vitamin D leads to dysregulation of immune responses.

Treatment for Vitamin D deficiency

Dietary supplementation with calcium and vitamin D may improve periodontal health, increase bone mineral density in the mandible, and inhibit alveolar bone resorption.[21] Vitamin D may help with periodontitis treatment not only because of its direct effects on bone metabolism, but also because it

may have antibacterial effects on periodontopathogens and decrease inflammatory mediators that lead to periodontal deterioration[5]

The major sources of vitamin D are sunlight (3,000 IU vitamin D3/5-10 min of mid-day, midyear exposure of arms and legs for a light-skinned Caucasian), and foods like oily fishes (100 to 1,000 IU/3.5 oz. vitamin D2 and D3 can be supplemented as capsules each containing about 400-5,000 IU/capsule.[5]

The treatment regimen for vitamin D deficient patients is as follows :50,000 IU of vitamin D2 or D3 weekly for 8-12 weeks as a repletion therapy. Once the initial repletion phase is complete, maintenance therapy should be followed:

1. 50,000 IU vitamin D2 or D3 every 2 weeks
2. 1,000-2,000 IU vitamin D3 daily
3. sunlight exposure for 5-10 min for Caucasians (longer times required for people with increased skin pigmentation) between the hours of 10 AM to 3 PM (spring, summer, and fall).[22]

Vitamin D supplementation and periodontitis

Various studies were conducted to know the effect of supplementing vitamin D for periodontally compromised patients. A study by Dietrich et al. stated that vitamin D may reduce the risk of gingival inflammation by its anti-inflammatory properties, as concluded from the study, the individuals with higher levels of 25(OH)D experienced 20% less bleeding on probing than those with low levels.[23]

One such study established the effect of Vitamin D supplementation on tooth loss, it was observed that Vitamin D supplementation per day for 3 years reduced the risk of tooth loss by 60%. One major limitation of the study was that calcium was also added to vitamin D supplements[24]. In another study, placebo-controlled trial there was improved periodontal health in participants who received Vitamin D supplementation than placebos. This study also had limitations in including calcium supplementation along with Vitamin D. There was very little information provided regarding the assessment of periodontal health in this study.[25]

In a study done by Miley et al.[26], periodontal status was analyzed among the individuals who take Vitamin D and calcium supplements, the supplement takers had shallower probing depths, less bleeding sites, lower gingival index values, fewer furcation involvements, less attachment loss, and

minimal alveolar crest height loss.[26] A study was carried out to assess the effect of presurgical Vitamin D status on periodontal surgery outcomes. The study revealed that daily calcium and Vitamin D supplements post-surgically caused greater resolution of infra-bony defects. Additionally, Vitamin D deficiency at the time of periodontal surgery negatively affects treatment outcomes for up to 1 year this indicates the importance of Vitamin D for post-surgical healing.[27]

Conclusion

Vitamin D plays an important role in maintaining overall health and also periodontal health. Vitamin D apart from its major role in the skeletal system, various studies have proven its role on the immune system, cardiovascular system, and also on cancer cells. Vitamin D plays a role in alveolar bone remodeling and also has immunomodulatory, anti-inflammatory, and antibiotic properties in the periodontal tissues. Hence, vitamin D serves as a boon for the management of periodontal disease.

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The Systemic Ripple: Exploring its Influence on Periodontal Health

Dr K Lakshmi Priya MDS
Assistant professor, Dept of Periodontology
E mail ID: *drlakshmipriya.perio@madch.edu.in*

Dr K Aishwarya BDS
Student
E mail ID: *drkaishwaryakrishnakumar@gmail.com*

Dr. R. Riduvarshini BDS
Student
E mail ID: *K.r.riduvarshini@gmail.com*

Dr N Ambalavanan MDS
Professor & Head, Dept of Periodontology
E mail ID: *drambal@gmail.com*

Introduction

A variety of systemic conditions spanning from hormonal changes during pregnancy and puberty to diseases involving dysfunction of our immune system, connective tissue disorders andleukaemia can present its symptoms in the oral cavity.[1] Nevertheless, it was rightly said that the mouth is the mirror to our body **(Fig 1).**[2] The state of health and functioning of the periodontium, which includes the Gingiva and the supporting structures of the teeth, are greatly influenced by aforementioned factors such as the blood circulation, hormonal fluctuations and immune responses.Changes or alterations in the overall health and well- being of an individual that impact any of these factors can be manifested as variations in periodontal well-being.[1]

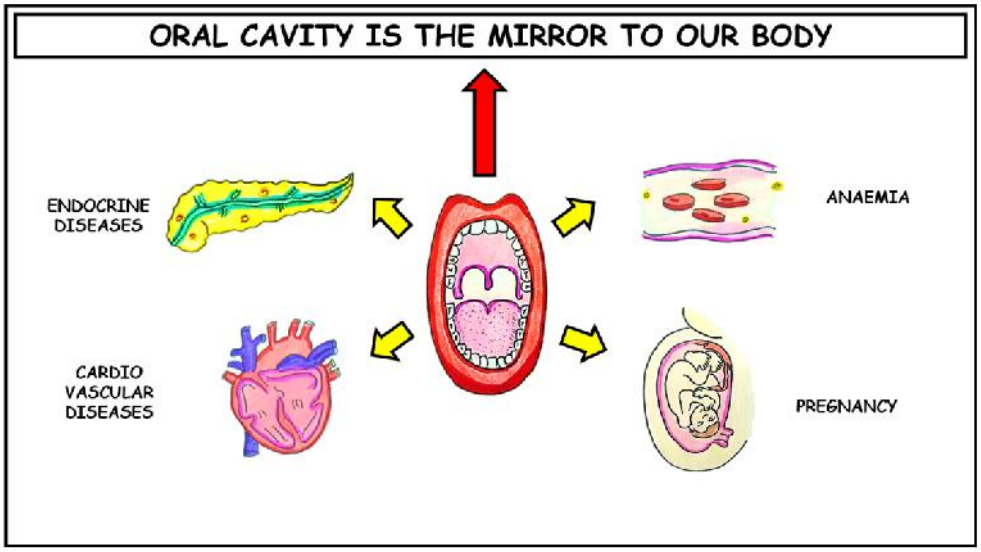

Figure 1: Diagrammatic representation depicting the systemic diseases and condition that reflects in the oral cavity

While this fact has been widely received, the primary emphasis in periodontology field still revolves around oral factors, risks related to oral factors and habits and impact/ outcomes of oral treatment. Nevertheless, recent scientific investigations also point out to the vice versa fact that periodontal diseases can also exert an influence on systemic health. And it can happen through two distinct mechanisms.The first mechanism directly revolves around the concept that plaque bacteria enter the blood stream leading to bacteraemia and second operates indirectly with periodontal disease inducing the production of inflammatory mediators like Prostaglandins and cytokines which in turn have distant effects on the body.[1]

There are various studies in literature supporting the evidence regarding the potential connections between systemic disorders and periodontitis and vice versa. Additionally, Periodontitis is also regarded as an independent risk factor for certain cardiovascular, endocrine and reproductive system related anomalies. Given this growing body of evidence highlighting the impact of various systemic conditionson the periodontium health it is imperative that we delve, a comprehensive literature review in this topic is essential**(Fig 1).**[3]

1. Diabetes mellitus

Diabetes mellitus, commonly known as diabetes, is a chronic metabolic disorder characterized by increased levels of blood glucose.While diabetes is primarily associated with disturbances in insulin production and utilization, emerging research has shed light on the intricate relationship between diabetes and periodontal health.The periodontal diseases may play an important role in the progression of periodontal disease and hence resolution of periodontal inflammation can have better glycaemic control in the management of diabetes.[4]

Periodontal diseases, such as gingivitis and periodontitis, are common oral health conditions that result from bacterial infections and inflammation of these tissues. Interestingly, there is a bidirectional relationship between diabetes and periodontal health, which influence the one and other in significant ways.Diabetes increases the risk of periodontal disease and complicates their management, while periodontal diseases, in turn, can worsen glycaemic control and exacerbate diabetes. Recognizing this interplay between oral health and diabetes is crucial for healthcare providers, as it underscores the importance of comprehensive care that includes both dental and medical aspects.[4]

The impact of diabetes on periodontium

1. Increased Susceptibility to Periodontal Diseases:

Individuals with diabetes are more prone to periodontal diseases. High blood glucose levels compromise the immune system's ability to fight infections, making diabetic patients more susceptible to bacterial invasion in the oral cavity **(Fig 2).** Consequently, the risk of developing gingivitis and periodontitis is substantially elevated in individuals with poorly controlled diabetes.[4]

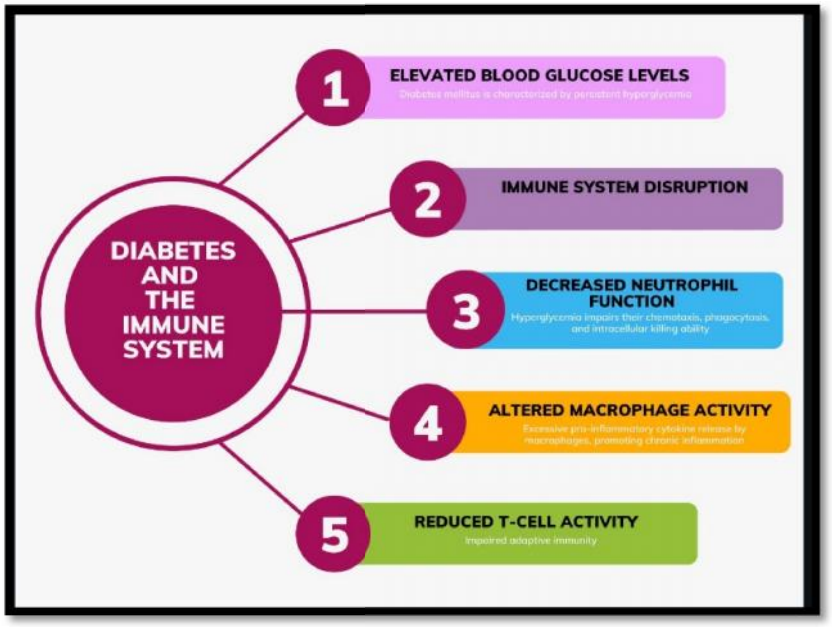

Figure 2: Diagrammatic representation depicting the effect of diabetes in our immune system

2. Altered Healing Response:

Diabetes can impair the body's ability to repair damaged tissues, including the periodontium. Even minor oral surgeries or routine dental procedures may pose a greater risk of complications in diabetic patients. Impaired wound healing and prolonged recovery times are common challenges faced by dental professionals when treating diabetic individuals **(Fig 3).**[4]

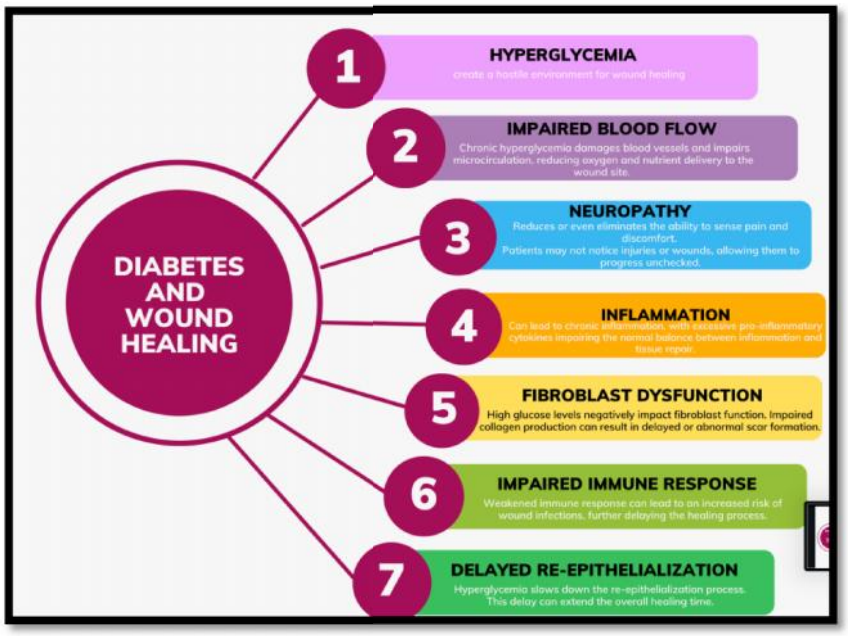

Figure 3: Diagrammatic representation depicting the effect of diabetes on wound healing

2 Effects of hormonal changes[4]

- Estrogen and Progesterone:

During pregnancy, significant increase in the production of estrogen and progesterone is natural. These hormones play a crucial role in promoting fetal development but also have systemic effects on the periodontium.

- Gingival Changes:

Elevated levels of estrogen and progesterone can lead to increased vascularization and permeability of gingival tissues. This makes the gingiva more sensitive to local irritants like dental plaque and can result in exaggerated gingival inflammation, a condition known as pregnancy gingivitis.

- Immune Response:

Hormonal fluctuations can also modulate the immune response. While this is important for fetal tolerance, it can weaken the maternal immune response to oral pathogens, allowing for an increased bacterial burden in the oral cavity.

Here's an overview of how androgens impact the periodontium:

1. Androgen Receptors:

- Androgens exert their effects by binding to androgen receptors present in periodontal tissues. These receptors are found on various cell types within the periodontium, including fibroblasts, osteoblasts, and immune cells.

2. Stimulation of Periodontal Cells:

- Fibroblasts:

Androgens can stimulate periodontal fibroblasts, which are responsible for collagen synthesis and tissue repair. This stimulation promotes tissue regeneration and wound healing in the periodontium.

- Osteoblasts: Androgens play a role in regulating bone metabolism. They promote osteoblastic activity, which is essential for maintaining the integrity of alveolar bone, a critical component of the periodontium.

3. Immune Modulation:

- Androgens can influence the immune response in the periodontium. They may have an anti-inflammatory effect by reducing the production of certain pro-inflammatory cytokines. This modulation can help regulate the immune response to periodontal pathogens and tissue damage.

4. Collagen Formation:

- Androgens promote the synthesis of collagen, a crucial structural protein in periodontal tissues. Collagen is responsible for the strength and resilience of the periodontium, and its production is essential for maintaining healthy gums and supporting structures.

5. Impact on Gingival Health:

- Androgens can contribute to gingival health by promoting tissue integrity and reducing susceptibility to gingival recession and inflammation.

6. Hormonal Imbalances:

- Hormonal imbalances, in conditions like polycystic ovarian syndrome (PCOS), can lead to elevated androgen levels in females hence can affect the periodontal tissues by, including gingival inflammation and increased susceptibility to periodontal diseases**(Fig 4).**[4]

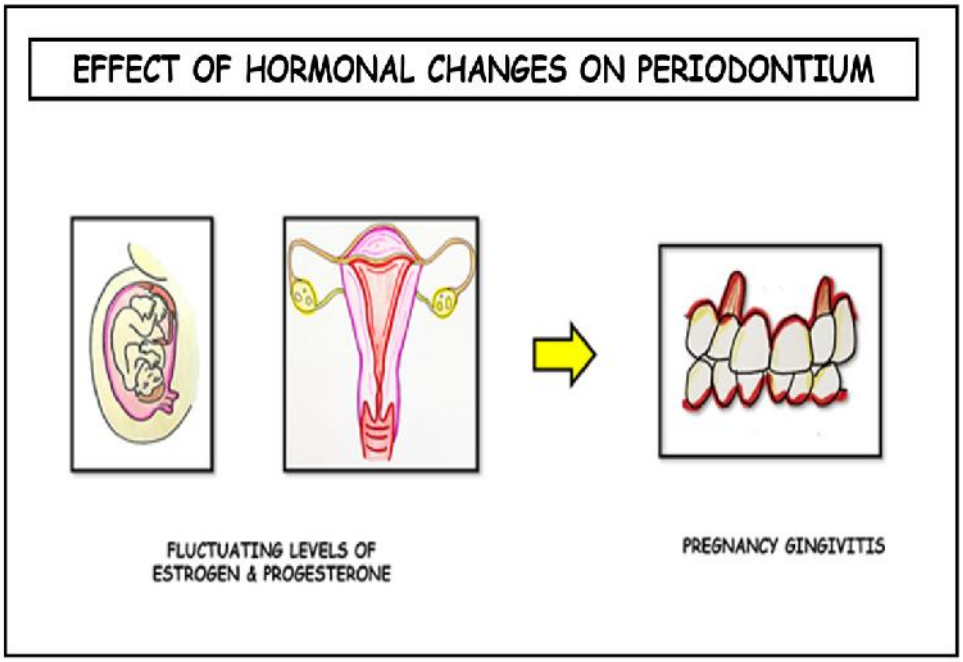

Figure 4: Diagrammatic representation depicting the effect of Hormonal changes on Periodontium

3. Hematologic conditions and immunodeficiencies

Leukaemia

Leukocyte (Neutrophil) Disorders

A) Leukaemia

They are malignant neoplasms involving precursors of white blood cells and exhibits the following characteristics.

- Extensive Bone marrow Replacement: Bone Marrow taken over by Multiplying Leukemic cells.
- Abnormal White blood cell count: Enormousimmature white blood cells in blood stream (**Fig 5)**.
- Widespread infiltration: Leukemic cells in various organs like Liver, spleen, Lymph nodes and other parts of the body.[4]

These can manifest oral and periodontal symptomssuch asbleeding, oral ulcerations, leukemic cell infiltration and infections **(Fig 5).** It is important to note that these oral signs often are associatedin cases with acute and sub-acute leukaemia when compared to chronic forms. This happens because the

leukemic cells are able to infiltrate the gingiva and also the alveolar bone occasionally leading to a condition called leukemic gingival enlargement. Conspicuously, this enlargement is not seen in edentulous individuals or in chronic leukemic patients. It is hence believed to result from accumulation of immature leukemic blast cells in gingiva adjacent to tooth surfaces that already harbour the bacterial plaque.[4]

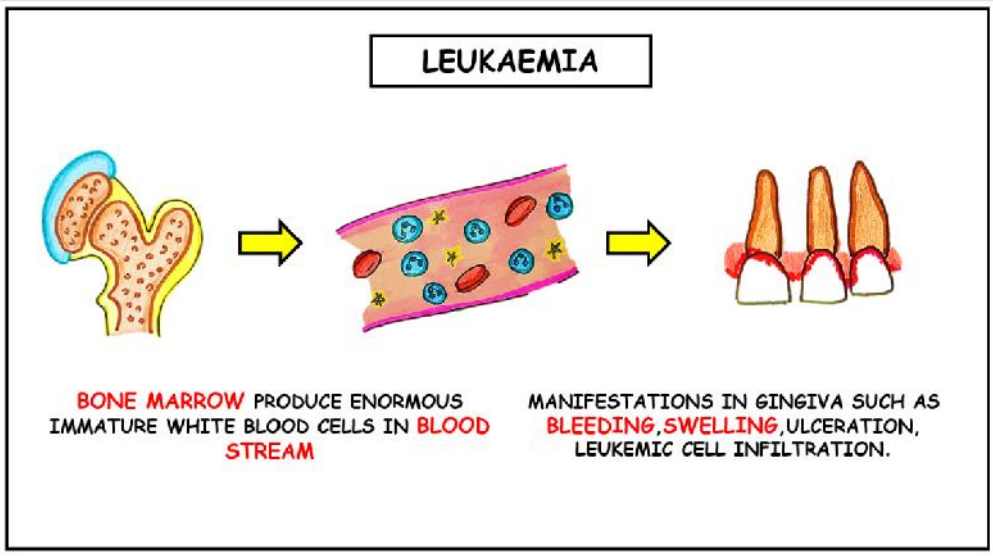

Figure 5: Depicting Leukaemia and its effect on the periodontium

Clinically leukemic enlargement presents with bluish-red and cyanotic appearance, along with rounding and tenseness of the gingival margin. In some cases, the cells can infiltrate the dermal and subcutaneous connective tissue referring to a condition called as leukaemia cutis, that leads to the formation of raised, flat macules and papules. Blood vessels of the affected areas are distended and there is decrease in the number of red blood cells (RBC's) and increase or infiltration of leukemic cells. The epithelium shows changes like thinning of the epithelium or hyperplastic growth. Patients diagnosed with leukaemia require specialized periodontal care.[4]

Gingival haemorrhage is a common finding in individuals suffering from leukaemia and can also manifest in patients not exhibiting any clinical signs of gingivitis **(Fig 5).** On the contrary this tendency to bleed from the gums can serve as an early indicator of Leukaemia and is primarily linked to thrombocytopenia, a condition where there is the replacement of bone marrow cells by leukemic cells and the disruption of normal stem cell function by these leukemic cells or their by-products. This bleeding tendency can also manifest

in the skin and throughout the mucosa leading to small red and purple spots known as petechiae which in turn can haveanadverse effect on chemotherapyin the management of leukaemia. [4]

Hence involving a dentist within a multidisciplinary healthcare team is crucial and dental treatment should be planned in such a way that they should adhere to carefully curated protocols with special attention to neutrophil and platelet counts. [5]

B) Leukocyte (Neutrophil) disorders

The importance of fully operational neutrophils in maintaining the periodontal health is underscored by the presence of neutrophil disorders that is associated with syndromes which manifests genetically**(Fig 6).**[6]It is mostly associated with inflammatory periodontal conditions and below we have outlined the periodontal attributes associated with certain clearly defined neutrophil irregularities**(Table 1).**[7]

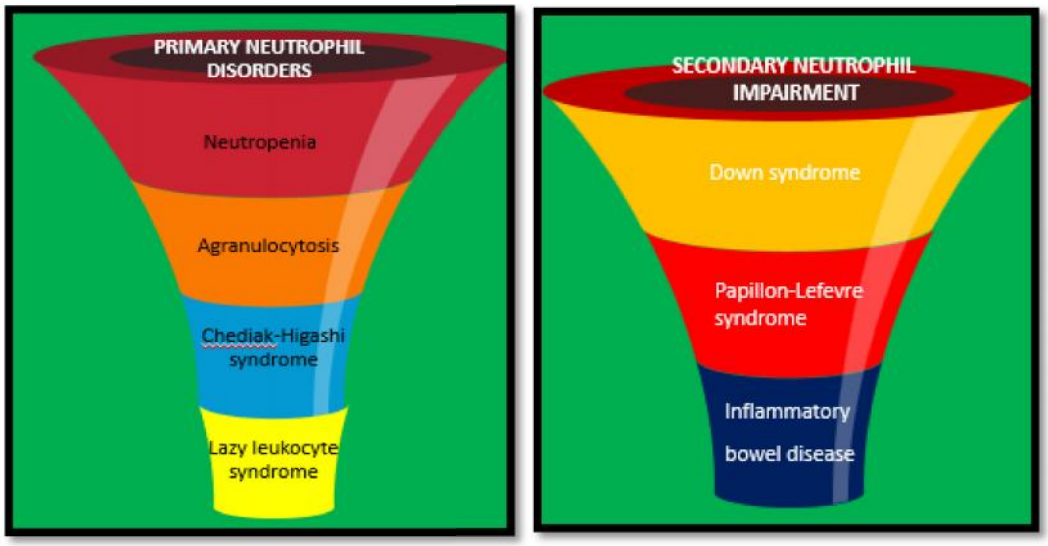

Figure 6: Primary and Secondary Neutrophil Impairment[6]

Table 1: Systemic conditions involving deficits in neutrophil function and its clinical manifestations.[7]

Systemic condition	Clinical Manifestations.
Chediak Higashi syndrome	Oral ulceration, Aggressive Periodontitis, recurrent infections, bleeding tendencies
Hyperimmunoglobulin E (Job syndrome)	Coarse facial skin, facial asymmetry, oral ulcerations, Gingivitis
Leukocyte adhesion deficiency I	Severe type of Periodontitis
Leukocyte adhesion deficiency II	Mental retardation, Periodontitis
Cyclic Neutropenia	Oral Ulcers, Gingivitis, Periodontitis
Papillon Lefevere syndrome	Severe aggressive periodontitis and premature loss of tooth
Lazy Leukocyte syndrome	Recurrent infections, Skin abscess, Gingivitis and periodontitis
Down syndrome	Acute necrotizing lesions, Periodontitis, Mental and growth retardation

4. The relationship between stress and psychosomatic conditionson periodontal health

Persistent stress and depression have been linked with periodontitis. Chronic stress has the potential to alter the composition of our normal commensal microbiota to pathogenic microbiome resulting in stress related dysbiosis**(Fig 7).** It is well received in literature that dysbiotic microbiome predisposes to various diseases. Periodontitis is an inflammatory condition that arises because of the aggregation of bacteria hence leading to inflammation which is characterized by a dysbiotic microbiota. There is a shift or decrease in coccoid and straight rod microbial population to more of motile organisms in diseased sites when compared to healthy sites.[8]

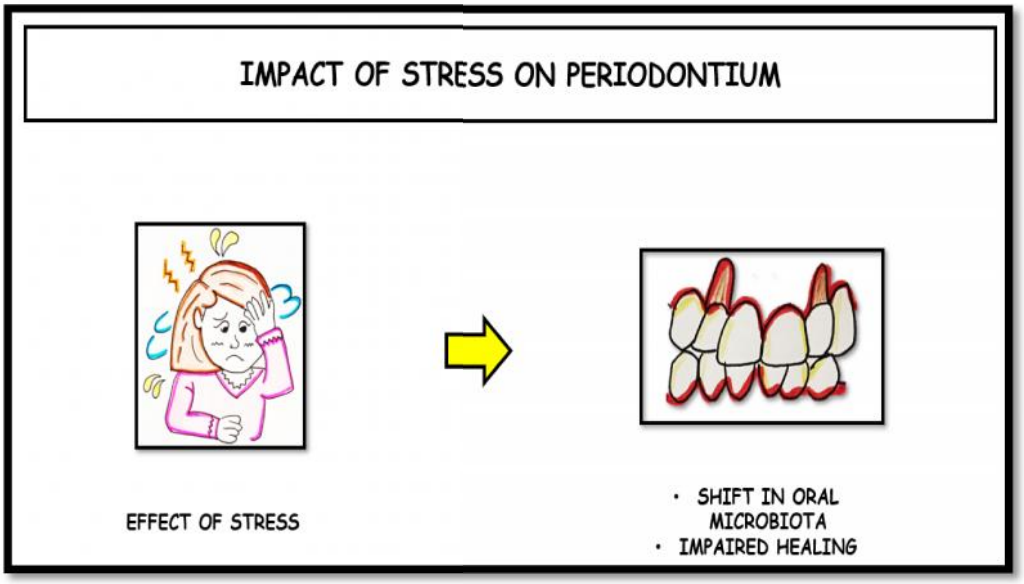

Figure 7: Depicting the impact of stress on periodontium

Furthermore,striking similarities can be found between stress and periodontitis in terms of inflammatory response and alterations in cytokine levels hence highlighting the possibility and necessity of addressing the shared pathogenic mechanism to effectively manage this complex multifactorial condition.[8] This involves implementation of a comprehensive set of interventions focussing at eliminating or at least controlling the risk factors, pathogenic mechanisms and clinical indicators associated with periodontitis. There is enough evidence in literature that supports and opens the door to a more thorough approach to manage and maintain periodontal health in individuals dealing with chronic stress and depression. [8]

5. Immunodeficiency diseases

Acquired immunodeficiency syndrome (AIDS)

AIDS is a condition characterized by a weak cell- mediated immune response and as consequence it results in systemic immune suppression leading to increased susceptibility to develop fungal, viral and bacterial infections and as well as malignancies that profoundly affect the overall health and well-being of the affected individual. Numerous studies have established the occurrence of necrotizing Ulcerative gingivitis accounting to 10% of individuals with HIV, while necrotizing ulcerative periodontitis accounts to about less than 5% classically affecting the patients with immunosuppression.[9]

In the early 1980's periodontal manifestations of HIV were clearly described that includes conditions like linear gingival erythema (formerly referred to as HIV-associated gingivitis), necrotizing ulcerative gingivitis, and necrotizing ulcerative periodontitis (formerly known as HIV-associated periodontitis). Oral manifestations of HIV encompass the following:[9]

- Oral candidiasis
- Oral hairy leucoplakia
- Kaposi's sarcoma
- Bacillary (Epithelioid) Angiomatosis
- Oral hyperpigmentation
- Atypical ulcers

6. Nutritional influences

It is well known that a majority of research findings support the fact that an individual's nutrional status has an effect on oral and periodontal health. Vitamins are broadly classified as Fat-soluble vitamins that includes A, D, E and K and water- soluble vitamins that includes the B-complex vitamins and C.

The fat- soluble vitamins mainly helps tomaintain epithelial cells integrity, skin and mucous membrane health and hence deficiency of this vitamin results in hyperkeratosis and hyperplasia of the gingiva. Vitamin D, the sunshine vitamin mainly helps in maintaining the calcium-phosphorous balance in the body and hence deficiency of this vitamin results in bone related disorders. The osteoclastic resorption of alveolar bone is increased in individuals with Vitamin D deficiency**(Fig 8).**Vitamin E is an important antioxidant and no reports have been established depicting the relationship between deficiency of vitamin E and periodontitis but, systemic vitamin E levels may help to enhance gingival wound healing**(Fig 8).**Vitamin K is very crucial for blood coagulation and hence deficiency of this vitamin results in increased tendency of gingival bleeding**(Fig 8).**

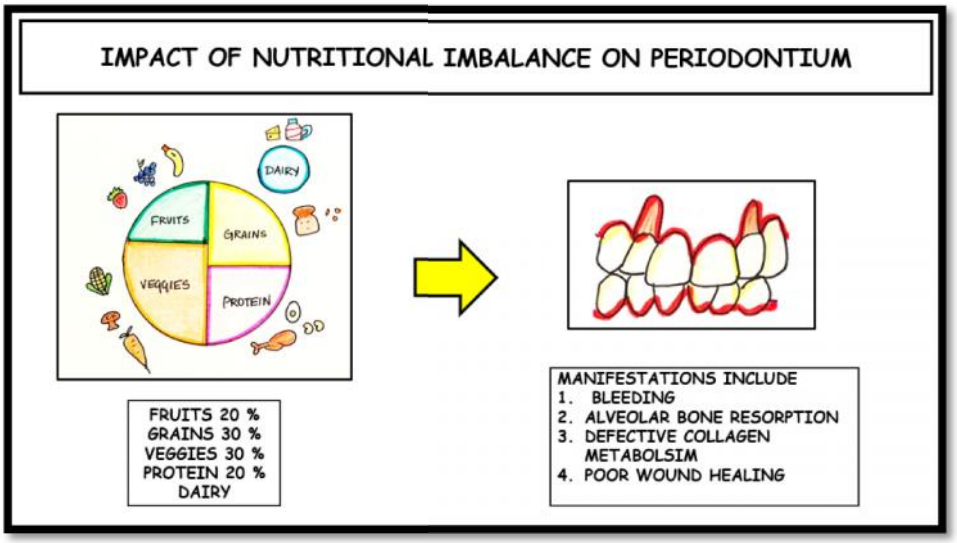

Figure 8: Impact of Nutritional imbalance on periodontium

The water- soluble B-complex family of vitamins consists of B1 (thiamine), B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B6 (pyridoxine, pyridoxal, pyridoxamine) B7 (biotin), B9 (folic acid), and B12 (cobalamins). This family of vitamins plays an important function in terms of cellular metabolism and repair. Deficiency of one of this family of vitamins rarely manifests as oral diseases but when there is deficiency is multiple B-complex vitamins clinical signs are noticed.[10]

Vitamin C (Ascorbic acid) is essential for the collagen synthesis and also acts as a ROS(Reactive oxygen species) scavenger to prevent oxidative stresshence decreasing the gingival inflammationinduced by ROS. The well-known periodontal manifestations of vitamin C deficiencyis scurvy which is bleeding, inflamed and painful gums.[10]The possible etiologic factors are: Within the periodontium, the low levels ofvitamin C namely the Ascorbic acid has its fair share of impact on the collagen metabolism hence affectingthe tissue's ability to undergo regeneration, repair and process of bone formation resulting in periodontal bone loss**(Fig 8)**. On the contrary, increasing ascorbic levels increases the chemotactic activity of neutrophils without affecting their phagocytic function. This shows that maintaining optimal level of ascorbic acid is essential to preserve the integrity of the periodontium and also aid in wound healing.[4]

Conclusion

Today, we have an immense understanding of the intricate communications between periodontal infections and our host systemic disease and its defence mechanisms. Various systemic diseases and genetic disorders have the power or potential to modify periodontal status hence affecting the oral health and also the quality of life. Therefore, characterizing those systemic conditions and understanding their impact on periodontal attachment apparatus offers valuable insights for appropriate diagnosis, drafting prognosis and also offer therapeutic strategies for the welfare of patients.

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Classification of foods - Carbohydrates, Proteins, Lipids, Vitamins and Minerals.

Dr.Karuna Kumari Jaddu[1] and Dr. B. Ramakrishna[2]
[1]HOD & Faculty, Department of Zoology, TSWRDCW, Mahabubnagar, Telangana, India
[2]Department of Zoology, Osmania University, Hyderabad, Telangana, India
E-mail: *drkarunajaddu007@gmail.com* & *raaam27@gmail.com*

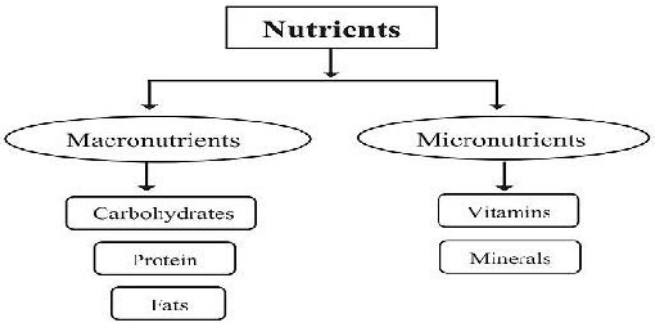
Introduction

Food is a basic and foundational part of our lives. Food plays a vital role for human existence just as the air we breathe and the water we drink. The food we eat is utilized in the body and assimilated substances are used for growth and maintenance of the tissue. People who eat right foods rich in nutrients enjoy their lives more, live longer, and are at a reduced risk of disease. Good nutrition is critical in preventing not only deficiency diseases, but also chronic diseases.

Nutrition is the study of nutrients in food, how the body uses them, and the relationship between diet, health, and disease. Nutritionists use ideas from molecular biology, biochemistry, and genetics to understand how nutrients affect the human body.

Nutrients

The substances which are present in the food and consumed in our body for its vital functions are called nutrients. According to the World Health Organization (WHO), these nutrients must come from food, and they are vital for disease prevention, growth, and good health. There are several constituents such as:



Classification of nutrients

Balanced Diet

The term "Balanced Diet" refers to a diet that provides all of the nutrients body needs, without too much of any one component. A Balanced diet contains all types of food in a right proportion. It means a balanced diet contains the right amount of carbohydrate, protein, fat or oil, vitamins, mineral salts and water depending on the desired needs of the body.

Malnutrition

Malnutrition is the eventual result of an imbalanced diet. Consuming too much or too little of any one nutrient, can cause malnutrition. Malnutrition can be so mild that a person suffers no ill effects. It can also be so severe that it causes serious illness or death. Malnutrition symptoms vary depending on the specific nutrient imbalance. Some possible symptoms include edema, chronic diarrhea, anemia, goiter, weight loss and decreased muscle mass.

Classification of Foods

There are more than 40 different kinds of nutrients in food and they can generally be classified into the following 7 major groups:

- Carbohydrate
- Proteins.
- Fats.
- Vitamins.
- Minerals.
- Dietary fibre.
- WaterWhy are they essential to our body?

Although each of the 7 major groups of nutrients performs different and unique functions in our body, they are all essential because they work together and contribute to our good health. The main functions of these major nutrients can be summarized as below:

Carbohydrates

Carbohydrates are a major source of energy of our body, and they come mainly from grains, such as rice and noodles. Besides, fruit, root vegetables, dry beans and dairy products also contain carbohydrates.

Proteins

Meat, fish, seafood, eggs, dairy products, dry beans and bean products are good sources of protein. Its major functions include building, repairing and maintaining healthy body tissues.

Fats

Fats can be found in foods such as meat, fish, seafood, dairy products, nuts, seeds and oils. Fats serve as an energy source. They prevent heat loss in extreme cold weather and protect organs against shock. They are responsible for making up part of our body cells and transporting fat-soluble vitamins such as vitamin A, D, E and K.

Vitamins

There are many kinds of vitamins from various food groups and they participate in different body metabolism such as maintaining healthy skin and hair, building bones and releasing and utilizing energy from foods. Vitamins can be classified into water-soluble and fat-soluble vitamins.

Minerals

Minerals are a group of essential nutrients which regulate many body functions such as fluid balance, muscle contraction and transmission of nerve impulses. Some minerals also contribute to body structure and build strong and healthy bones, such as calcium

Dietary fibre

Dietary fibre is the indigestible part found in plant. It helps stablise blood sugar, promote gastrointestinal health and prevent constipation. Dietary fibre can be classified into soluble and insoluble fibre.

Water

Water is the most abundant substance in human body and is also an essential nutrient to maintain our health. The major functions of water include regulation of body temperature, production of body fluids, transportation of nutrients and removal of waste products.

Although each nutrient performs different functions in our body, they all work together and contribute to our good health.

Food can be classified in accordance to their chemical property, to their function, to their essentiality, to their concentration and to their nutritive value.

CLASSIFICATION OF FOODS	
Based on	Classified Food
a.Chemical nature	Carbohydrate, Protein, Fats, Vitamins, Minerals,Dietary fiber,Water
b.Functions in the body	Energy giving, Body Building and Protective
c. Chemical properties	Organic and Inorganic
d. Mass	Macro Nutrients and Micro Nutrients
e.Origin	Plant Foods and Animal Foods
f. Nutritive Value	12 categories

a) According to the chemical nature

- Carbohydrates
- Proteins
- Fats (Lipids)
- Vitamins
- Dietary Fiber
- Water Minerals.

b) According to their function in the body

Energy giving foods

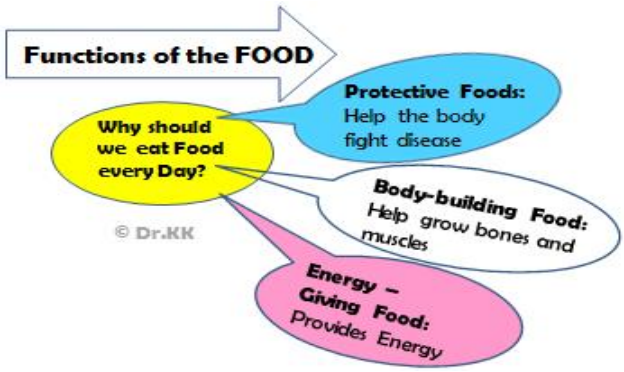
The carbohydrates, fats and the protein are considered as calorie nutrients as they give energy, so that the body can perform the necessary functions. Rice, chapatti, bread, potato, sugar, oil, butter and ghee are examples of energy giving foods.

Body building foods

Foods such as proteins, fats and carbohydrates are also called as body-building food. They are the nutrients that form body tissues, muscles and bones. Fish, meat, chicken, eggs, pulses, nuts and milk are some body building foods.

Protective foods

Vitamins and minerals are the nutrients that function to regulate body processes and help the body to fight diseases. They protect us from various diseases. Fruits and vegetables are some examples. Therefore we must eat these regularly.



c) According to chemical properties

Organic: Nutrients that contain the element of carbon are called as organic nutrients.

Inorganic: Nutrients that do not contain carbon element are called as inorganic nutrients.

The organic nutrients include carbohydrates, lipids, proteins and vitamins. Water and minerals are inorganic.

d) According to its mass depending on the quantity necessary for cells and organisms are classified as

Macronutrients: Macronutrients are required in large quantities daily. Proteins, carbohydrates and fats are macronutrients. They are the basis of any diet.

Micronutrients: Micronutrients are needed in small quantities (usually in amounts less than milligrams). These nutrients are involved in regulating metabolism and energy processes.They are vitamins and minerals.

e) According to its origin

Depending upon the origin of food it has been classified as animal food sources and plant food sources.

f) According to its nutritive value

• Cereals and millets• Pulses• Nuts and oil seeds•Vegetables•Green leafy vegetables

• Non-leafy vegetables• Roots and tubers• Fruits• Milk and milk products

•Animal foods—meat, fish, liver, egg etc• Carbohydrate foods• Condiments and spices.

Classification of foods based on the chemical nature:

I. Carbohydrates:

Carbohydrates are the chief sources of energy (Providing 4 Kcals/gm). Carbohydrates are cheap and readily available from food. There are three different kinds of carbohydrates. They include starch, sugar, and fibre. We do not get calories from fibre because our bodies do not break down fibre during digestion.

Classification of Carbohydrates

Monosaccharides: these are the simplest form of carbohydrates containing simple sugar molecule. Example: Glucose, Fructose and Galactose.

Disaccharides: These carbohydrates composed of two units of Monosaccharides. Example: Sucrose, Lactose and Maltose.

Polysaccharides: these are the complex sugars containing numerous units of monosaccharide molecules. Example: Glycogen, Cellulose and Pectins.

Daily requirements

Carbohydrate intake should be in the range of 300-500gm (50%- 70%) out of the total energy intake for adults and 40-60% for children.

Sources: All sugars, jaggery, honey, pulses, whole grains, cereals, grains, rice, fruits, milk, yogurt, beans, roots and tubers such as potatoes, beet root etc.

Functions of Carbohydrates:

- Supplies energy
- Protein sparing function
- Oxidation of fats
- Regulating blood glucose
- Facilitates bowel movements

Digestion and absorption of Carbohydrates:

Salivary amylase aids digestion of starch in the mouth. Most of the digestion of carbohydrates takes place in the small intestine. Carbohydrates are absorbed into the blood stream as glucose, Galactose and fructose. By way of the capillaries of the villi, the simple sugars enter the portal circulation and transported to the liver.

II. Proteins

Proteins are the indispensible constituents of the diet. Proteins are made up of amino acids. Amino acids are needed for replacement and growth of the body parts.

Amino acids are classified as essential and non essential amino acids. Essential amino acids cannot be synthesized by the body and must be taken through foods whereas non essential amino acids can be synthesized by the body.

Daily requirements

The ICMR recommends 1gm of protein/ Kg of body weight for adults.

The amount of protein should be increased for children, pregnant and lactating mothers by 1.5- 2 g/kg body weight.

Sources of Protein

Animal sources: eggs, milk and milk products, fish, meat.

Plant sources: pulses, cereals, dry fruits, beans nuts etc.

Functions of Proteins

• Protein helps in synthesis of enzymes, immunoglobulin, plasma proteins and hormones in the body

• Protein helps in growth and repair of body tissues

• Proteins are secondary sources of energy during deficiency of carbohydrates and fats. (Provides 4 Kcals/gm) • Proteins help in forming haemoglobin • Proteins help in antibody formation

Digestion and absorption: The hydrolysis of proteins in the gastro intestinal tract is accomplished by proteases secreted in gastric juice and pancreatic juice and also by proteases present in the intestinal mucosa.

III Fats: Lipids

Fat is deposited as adipose tissue in the body and perform essential functions in the body. Fats are composed of fatty acids and contain oxygen, carbon and hydrogen.

Classification: Fats are classified in to two types: saturated fat and unsaturated fat.

a) Saturated Fat: These have full number of hydrogen atoms. These are from animal sources. Example: butter, Ghee and vanaspati oil.

b) Unsaturated Fat: These contain one, two or more double bonds of fatty acids. These are extracted from vegetable sources. Example: Groundnut oil, soyabean oil, sunflower oil.

Daily Requirements

15-20% of total calorie requirements should be from fat

Sources

Animal sources: Fish, egg, meat, milk and milk products.

Plant sources: *oil seeds (Groundnut, mustard, cotton seed and coconut oil), nuts.*

Functions

• Supplies energy (9 kcals/ gm),• Improve the palatability of food (flavor and taste)

• Supports body organs like liver and kidneys

• Provides insulation and thermoregulation against cold

• Provides essential fattyacids which helps in growth, promotion and maintenance of skin integrity

• Helps in formation of hormones in the body. • Helps in transportation of fat soluble vitamins

Digestion and absorption

There is no digestion of fats in mouth and very little in stomach. In the small intestine, presence of fat stimulates cholecystokinin and secretin which further stimulates pancreatic juice and bile. Food mixes with bile and emulsified. Pancreatic lipase hydrolyses and yields fatty acids and monoglycerides. These pass to small intestine and combined with intestinal lumen and absorbed in to blood stream.

IV. Vitamins:

vitamins are complex chemical substances required by body in very small amounts. Vitamins in food are for the protection and regulation of body functions. • Vitamins are divided in to two major groups

- Fat soluble vitamins- Vitamin-A, D, E and K
- Water soluble vitamins- Vitamins of B group and vitamin C

A.Fat soluble vitamins:

1. Vitamin-A

The chemical name is Retinol. Found in foods of both plant and animal origin.

Daily requirements: 0.4-1 mg Sources

Green leafy vegetables and yellow orange fruits and vegetables like mango, papaya, pumpkins and carrots are good sources of β-carotene.

Butter, whole milk, egg, liver and fish are richest sources.

Functions

• Necessary for the health of the eyes.

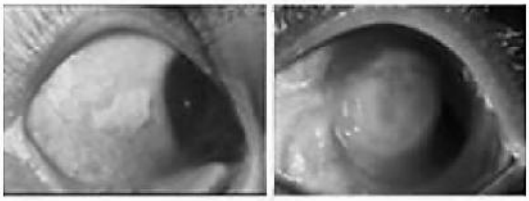
• Maintenance of the normal epithelial tissues of the body.

• Necessary for growth and proper utilization of protein

Deficiency Syndrome

• Decreased resistance to infection • Dry scaly skin

- Night blindness (Inability to see in dim light)
- Xerophthalmia- dry eye
- Bitot's spots- Greyish, rough and raised patches on conjunctiva
- Keratomalacia- Softening of the cornea.
- Stunted Growth



Bitot's spot Keratomalacia

2.Vitamin-D

Vitamin D is synthesized by sunlight. Vitamin D is essential for bone growth.

Daily requirements: 400 IU Sources

Generated in the skin by action of ultra violet rays of sunlight

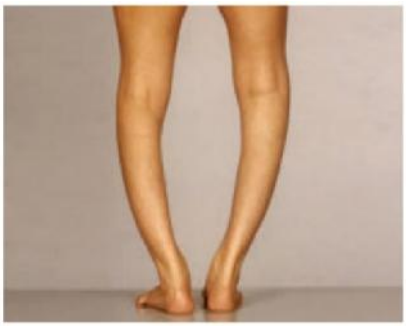
Food sources are milk, butter, cheese, egg, fish and fish liver oils, and foods which have been fortified by addition of vitamin D.

Functions

•Increases intestinal absorption of calcium and phosphates

•Mineralization of bones

Deficiency Syndrome



Rickets in children- Bony deformities in growing children due to defective mineralization of the growing bones. Bones become soft and bend under pressure.

Osteomalacia- Generalized bone pain in adults especially in women.

3. Vitamin E:

Vitamin E is an antioxidant and formed up of chemical substance called tocopherols.

Daily requirements: ICMR recommends 0.8 mg/ g of essential fatty acids.

Sources: Milk, oils, eggs, leafy vegetables, papaya, grains, nuts.

Functions

• Antioxidant (Prevents the formation of oxidative free radical)

•Co factor in electron transport

• Prevents or delays the ageing

Deficiency syndrome: Sterility, muscle wasting with weakness

4. Vitamin K

It is otherwise called as antihemorrhagic vitamin.

Daily requirements: WHO suggested RDA of 55 µg per day for adults.

Sources: Green leafy vegetables, cereals, fruits. Synthesized by bacteria in gut.

Functions:

Important component in blood coagulation

Helps in formation of blood clotting factors

Deficiency Syndrome: Alteration in blood clotting mechanism.

B. Water soluble vitamins:

These vitamins are essential for many metabolic functions of the body.

1.Vitamin B_1- Thiamine:

Thiamine is essential for the normal metabolism of carbohydrates and fats. It is necessary for the transmission of nerve impulses.

Daily requirements: 0.5 to 2 mg

Sources

whole grain cereals, wheat, ragi, pulses (dhal), vegetables and potatoes, green leafy vegetables. Meat, fish, liver and eggs.

Functions

Helps in carbohydrate utilization.

Maintenance of appetite and digestion.

Deficiency Disorder

Beriberi- condition in which there is a severe muscle wasting, growth retardation in children, neurological disturbances and frequent infection.

2.Vitamin B_2

Vitamin B_2, also known as riboflavin, is one of the eight B-complex vitamins. It is essential for the health of skin and for normal vision.

Daily requirements: 0.6 mg/ 1000 Kcal

Sources:

Riboflavin is found in eggs, nuts, dairy products, meats, broccoli, sprouts, wheat germ, wild rice, mushrooms, soya beans, green leafy vegetables and whole grain and enriched cereals and bread. Riboflavin also synthesized by bacteria in intestine.

Functions

It helps the body break down carbohydrates, proteins and fats to produce energy.

It allows oxygen to be used by the body.

Deficiency Syndrome

- Glossitis- Ulceration of the tongue.
- Angular stomatitis- Cracks at the corner of the ps.
- Corneal ulceration.

3. Vitamin B_3

Niacin is one of the B-complex nutrients; it can be synthesized in body.

Daily requirements: 10-15 mg

Sources

Found in appreciable amounts in liver, yeast, meat, legumes, peanuts and whole cereals.

Foods that are good sources of tryptophan are animal protein and vegetable protein.

Functions

Helps in normal functioning of skin, intestinal tract and nervous system

Deficiency Syndrome

Pellagra- Three conditions are

- Dermatitis -dark, dry and scaly skin
- Diarrhea- due to atrophy of intestinal wall
- Dementia-Memory Loss

4. Vitamin B_5- Pantothenic acid

Anti dermatitis factor

Daily requirements: 10mg

Sources

Eggs, liver, yeast, many fish and vegetables.

Functions

•Necessary for metabolic functions

Deficiency Syndrome

Dermatitis, hair loss.

5.Vitamin B_6

This vitamin $B_6$is otherwise known as pyridoxine. It is stored in muscle but found in tissues throughout the body.

Daily requirements: 1.5-2 mg for normal adults.

Source

Whole grains, legumes, bananas, potato, liver, kidney and other meats, fortified breads and cereals. Sunflower seeds, soya beans, walnuts and yeast are the richest sources of pyridoxine among plant foods.

Functions

- Production of red blood cells
- It is readily absorbed from intestines
- Improves immunity
- Improves nervous system function
- Reduce muscle spasms, cramps and numbness
- Maintains proper balance of sodium and phosphorous in the body.

Deficiency Syndrome

Anaemia, nervousness, insomnia, oedema (Water retention), mental depression. Muscle weakness, tooth decay.

Arm and leg cramps, Skin lesions and skin disorder.

6.Vitamin B_7

Otherwise known as Biotin. It is associated with carbohydrates metabolism.

Daily requirements: Traces

Sources

Egg yolk, liver, kidney, tomatoes, vegetables, legumes and cereals.

Functions

It is needed for protein and fatty acid synthesis

Deficiency syndrome: Dermatitis, hair fall.

7. Folic acid

Vitamin B_9 includes both folate and folic acid and is important for several functions in the body.

Daily requirements: 0.4mg

Sources

- Fish, mutton, liver, egg, chicken, green leafy vegetables, pulses, lentils, beans, sunflower seeds, beets, broccoli, spinach, orange juice, tofu, fish, meat, fortified cereals, milk, cheese, eggs, oysters, crab etc.,

Functions

- Folic acid helps the body to convert carbohydrates into glucose, which is used to provide energy.
- Folic acid helps in building of antibodies which prevent and heal infections.
- Regulates blood cells formation.

Deficiency Syndrome

- A recent study connected folic acid deficiency with autism
- Megaloblastic anemia
- Sterility
- Low birth weight babies
- Congenital defects in the child- cleft lip and cleft palate.

8. Vitamin B_{12}-Cyanocobalamine

This vitamin is destroyed by heat.

Daily requirements: 1-3 µg

Sources

Foods of animal origin, also synthesized by bacteria.

Functions

- DNA Synthesis
- Stimulates and promotes maturation of RBC's

Deficiency Syndrome

- Pernicious anemia
- Infertility
- Neurological and mental disturbances

9. Vitamin C

Vitamin C is also known as ascorbic acid.

It is an antioxidant and water soluble vitamin.

Daily requirements: up to 40 mg

Sources: Citrus fruits (amla, guava, lemon, orange, tomato), green leafy vegetables.

Functions

- Helps in wound healing
- Prevents bleeding
- Antioxidant

Deficiency Syndrome

Scurvy- Painful swelling of gums and joints. Multiple hemorrhages specially in gums, skin and mucus membrane Delayed wound healing.

Vitamin	Constituent	Deficiency Diseases	Sources
Vitamin A	Retinol, Retinoic Acid, BetaCarotene	Nightblindness,Healing epithelial cells,Normal development of teeth and bones	Carrots, Papaya, Milk, Cheese, Fish Liver Oil, Green Vegetables etc.
Vitamin B1	Thiamine	Beriberi	Brewer's Yeast, Whole Grain, Oatmeal, Legumes, Peanuts, Dried Soybean, Sunflower Seeds etc.
Vitamin B2	Riboflavin	Ariboflavinosis	Beef Liver, Lamb, Milk, Mushroom, Spinach, Almonds etc.
Vitamin B3	Niacin or Nicotinic Acid	Pellagra	Tuna, Chicken, Turkey, Mushrooms , Bacon, Broccoli, Veal etc.

Vitamin B5	Pantothenic Acid	Acne, Paresthesia	Chicken Liver, Sunflower Seeds, Salmon, Avocados, Corn, Broccoli, Mushroom etc.
Vitamin B6	Pyridoxine, Pyridoxal, Pyridoxamine	Dandruff-like eruptions, Pink eye, Epilepsy	Potatoes & other starchy vegetables, Fruit (other than citrus) etc.
Vitamin B7	Biotin	Growth & Neurological Disorders in Infants	Raw Egg Yolk, Liver, Peanuts, Yeast, Whole-wheat Bread, Cheddar Cheese, Pork etc.
Vitamin B9	Folic Acid	Macrocytic Anaemia, Birth Defects	Dark Leafy Greens like Spinach, Asparagus, Broccoli, Citrus Fruits, Beans, Peas, Lentils, Avocados etc
Vitamin B12	Various Cobalamins	Macrocytic Anaemia, Memory Loss, Pernicious Anaemia, Mania, Psychosis, Paralysis	Seafood, Beef, Chicken, Eggs etc.
Vitamin C	L-Ascorbic Acid	Scurvy	Amla, Guava, Chillis, Kiwi, Broccoli, Orange, Papaya, Lemon, etc.
Vitamin D	Calciferol (D2) & Cholecalciferol (D3)	Rickets, Osteomalacia,Needed for absorption of calcium from small intestines,Calcification of the skeleton	Sunlight, Mushrooms, Alfalfa, Fish Liver Oils, Cooked Egg Yolk, etc.

Vitamin E	Tochopherols & Tocotrienols	Red Blood Cell Destruction, Ataxia, Retinopathy, Peripheral Neuropathy, Reproductive Failure	Wheat Germ Oil, Canola Oil, Sunflower Oil, Almond Oil, Hazelnuts, Peanuts etc.
Vitamin K	Phylloquinone (K1), Menaquinone (K2)	Lack of Clotting of Blood, Lack of Tissue Renewal	Green Leafy Vegetables etc.

V.Minerals:

These are the inorganic compounds in micro quantities which are essential for many vital functions of the body. The minerals constituent of the body amounts to 4.3 to 4.4 %, largely in the skeleton.

General Functions of Minerals

- As constituents of hard tissue. (eg.) calcium and phosphorus in bone and teeth.
- As constituents of soft tissue. (eg.) Sulphur and phosphorus.
- As constituents of substances assisting in the regulatory function of the body (eg.) salts in solutions influence nerve and muscle action.
- Some of the essential mineral salts are sodium, potassium, calcium, phosphorus, magnesium, iron and iodine.

1.Calcium

Calcium is most important for children and pregnant women.

Calcium is important component in bones and enamel of teeth. 99% of body calcium is found the bones.

Sources: milk and its products, green leafy vegetables, bones of meat, fish, pumpkin, coconut, dry fruits, cereals.

Daily requirements: 400- 500 mgm

Functions

- Formation and maintenance of bones and teeth
- Coagulation (Thickening) of blood

- Muscle contraction.

Deficiency Disorders:

Deficiency of calcium in the body precipitates rickets in the children and osteomalacia in adults.

2. Sodium

Sodium is essential for many body activities. It is taken in the diet as salt.

Sources: Common salt, sodium chloride is also found in certain foods like fish, meat, eggs and seasoned foods.

Daily requirements: 8-10 gms.

Functions

- It helps in transmission of nerve impulses
- Maintenance of body fluids and electrolytes balance
- Smooth functioning of nerves, muscles and body cells

3.Potassium

Most of the potassium present inside the cells.

Sources: Fresh vegetables, citrus fruits, milk, guava and amla.

Functions

- Involved in various biochemical activities of the cells.
- Transmission of nerve impulses.
- Maintenance of electrolyte balance and contraction of muscles.

Daily requirements: 2-5 gms

4. Phosphorous

Most of the phosphorus present in the bones as inorganic form. Few amount resent inside the cells.

Sources:

Whole grain cereals and flours, legumes, oatmeal, cheese, nuts, fish

Functions

- Gives rigidity to bones and teeth

- Regulates pH of the blood and urine
- Important in energy metabolism
- Phosphorus compounds are necessary for carbohydrate metabolism and for the calcification of bones and teeth
- Needed for transport of fatty acids

5. Iron

The amount of iron present in the adult human body is very small, but it is very important substance and essential for the maintenance of life. 75% of total body iron present in the blood.

Sources: Liver, meat, fish, eggs, cereals, pulses, green leafy vegetables, dry fruits, jaggery, certain beans.

Daily requirements: 25-40 mg

Functions

- Component of hemoglobin and myoglobin
- Helps in oxygen transport and cellular respiration

Deficiency Disorders:

- Iron: Reduced level or lack of iron causes anemia, certain hormonal changes in women.

6. Iodine

Iodine is considered as an important for maintaining metabolic rate.

Sources: Iodized salt, salt water fish, milk, meat cereals and green leafy vegetables.

Fortification of common salt with potassium iodate is a recommended method of making iodine easily available.

Daily requirements: 01.05 mg

Functions of Iodine

- Iodine is an important constituent of thyroxine, the hormone secreted by the thyroid gland
- Iodine is responsible for the regulation of physical growth.
- Deficiency Disorders: Lack of iodine in the body leads to enlargement of thyroid gland called as goiter(Simple Goiter).

7. Magnesium

Human body contains about 25gms of magnesium. Half of it is present in bones and in combination with phosphate and carbonate and about 1/5th in soft tissues.

Functions

Constituent of bones and teeth, coenzymes in general metabolism, smooth muscle action.

Sources of Magnesium: Dairy products (excluding butter) fresh green vegetables, meat, nuts, sea food and legumes are good sources of magnesium.

Other inorganic elements

9. Copper: Functions with iron in the formation of haemoglobin.

10. Manganese: Has a similar effect though less marked than copper.

11. Cobalt: It is present in vitamin B_{12} & is also necessary for the formation of haemoglobin.

12. Zinc: Is found mainly in pancreatic tissue and may have an important part to play in the storage of insulin in the gland.

Clinical Significance of Minerals

Nutrient	Deficiency disease	Source
Essential Fatty Acids	Omega 3 (Alpha Linolenic Acid) and Omega 6 (Linolenic Acid)	Several bodily processes afflicted, Skin Ailments
Calcium etc.	Osteoporosis, Hypocalcemia, Ostopenia, rickets in the children and osteomalacia in adults.	Milk and Milk Products, Eggs, Wheatgrass
Sodium	Cognitive Impairment, Headaches, Nausea, Seizure, Coma, Electrolytic Imbalance	Salt, Fish, Meat, Vegetables etc.
Potassium	High Blood Pressure, Arrhythmia, Muscle Weakness, Myalgia, Muscle Cramps,	Meat, Milk, Fruits, Vegetables, Whole Grains etc.

	Constipation, Respiratory Depression, Paralysis	
Magnesium	Deterioration of Metabolism & Cellular Functioning, Heart Attacks, Insulin Resistance	Nuts and Seeds, Green Vegetables, Dark Chocolate, Whole Grains etc
Phosphorous	hypophosphatemia , rickets in children and osteomalacia in adults	Meat, Fish, Poultry, Eggs, Milk, Bananas etc.
Iodine	Goitre, Cretinism, Deterioration of Metabolism & Cellular Functioning	Iodised Salt, Sea Food, Green Vegetables, Raw Milk, Eggs etc.
Chlorine	alkalosis	Salt, Milk, Meats, Vegetables etc
Protein	Kwashiorkor	Meat, Seafood, Eggs, Pulses & Legumes, Milk & Milk Products etc.
Protein-Energy (Malnutrition)	Marasmus.	Grains, Pulses & Legumes, Meat, Milk & Milk Products, Eggs, Seafood etc
Iron	anemia	Liver, Leafy vegetables ,Jagerry etc

VI. Dietary fibre:

The carbohydrates (E.g, pectin, cellulose, hemicellulose) and some non carbohydrates substances (e.g. lignin) are collectively called as dietary fibre. Fibre is found in vegetables, fruits and grains. It resists digestion.

Significance of Fibre

High fibre diet plays an important role in prevention and management of constipation (Inability to pass motion/ lack of bowel movement)

Fibre also helps to reduce blood cholesterol

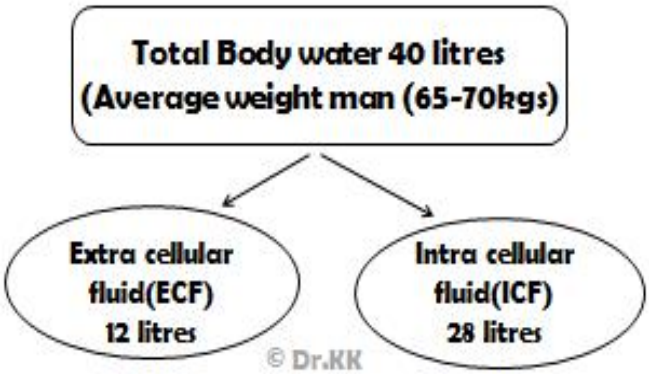
Helps to prevent blood glucose level after food (Post prandial blood glucose).

VII. Water

Water is an important component with diet as it performs many vital functions in the body and hence is a part of balanced diet. Water makes up to 70% of total body weight inhuman beings. Water should be taken in enough quantities to prevent dehydration.

Functions of Water

- Water is a major constituent of blood and tissue fluid
- It helps in transport of many substances from one compartment to another
- Provision of the moist internal environment required by all living cells
- Participation in all the chemical reactions occurring extracellularly and intracellularly
- Regulation of the body temperature



Distribution of Body Water

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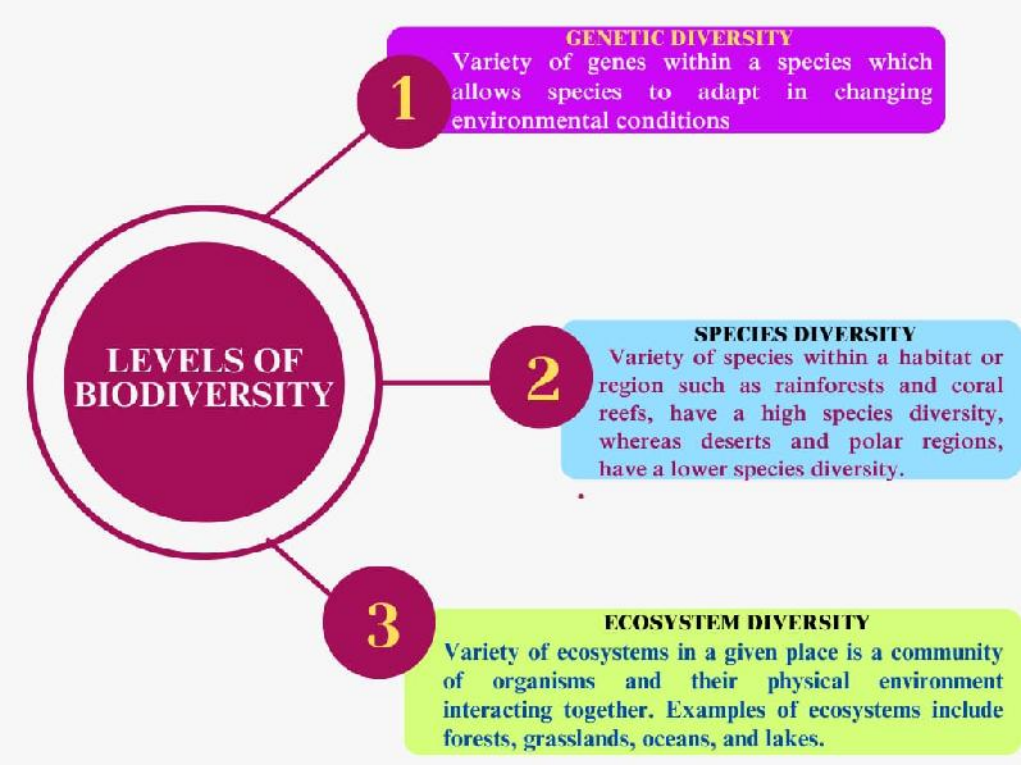
Biodiversity and Hotspots of Madhya Pradesh

Dr. Sangeeta Sarkhel
Assistant Professor
PG Department of Zoology
Mata Gujri Mahila Mahavidyalaya (Autonomous), Jabalpur

Introduction

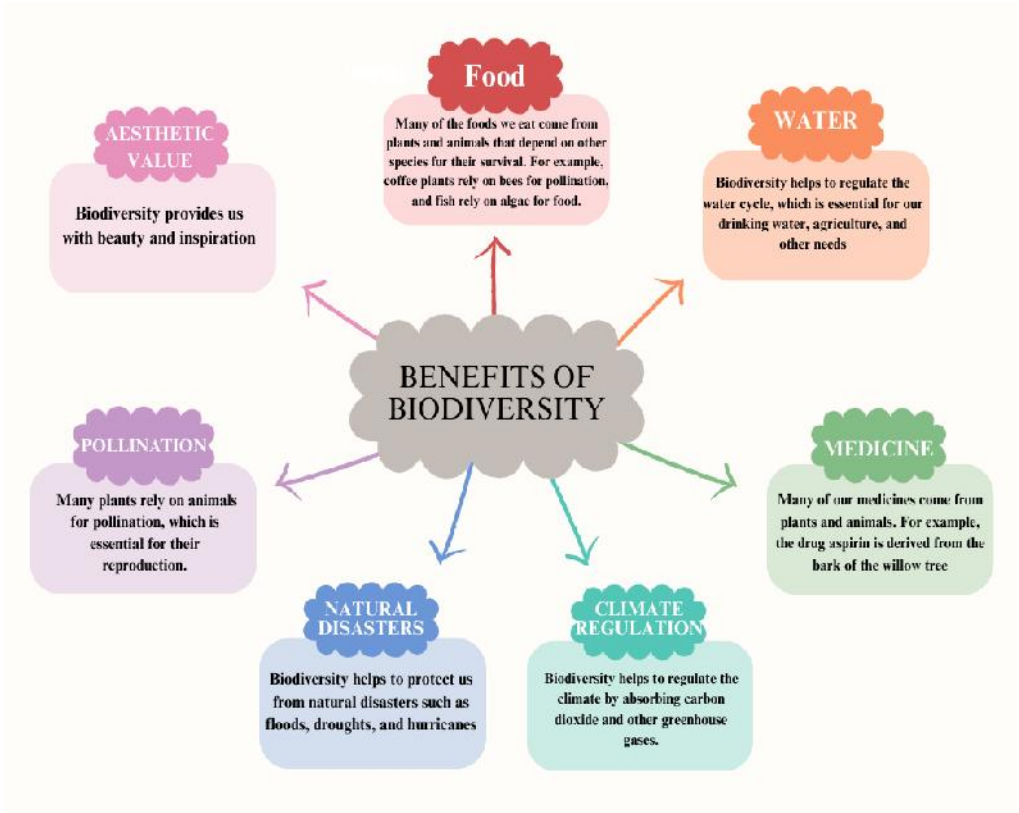
Biodiversity is the variety of life on Earth. It includes all living things, from the smallest bacteria to the largest blue whale, as well as the ecosystem they live in. Biodiversity can be measured at three levels:, genetic diversity, species diversity and ecosystem diversity .



Biodiversity is important for a number of reasons. It provides us with the food we eat, the air we breathe, and the water we drink. It also helps to regulate the climate, protect us from natural disasters, and provide us with medicines and other resources.

The impact of landscape features on genetic population structure, through microsatellite and mitochondrial DNA analysis, among populations found across the particular area. Genetic variation was not a function of local abundance but it correlated significantly with habitat (Costa et al, 2021).

Some of the benefits of biodiversity:



Biodiversity is under threat from a number of human activities, including deforestation, pollution and climate change. These activities are the cause of destruction of habitats, killing species and ecosystem disturbance. It is important to conserve biodiversity for the sake of our own survival. Manes et al. (2021) reviewed over 8000 projections of impact of climate change on biodiversity in 232 studies, including 6116 projections on endemic, native and introduced species in terrestrial (200 studies), freshwater (14 studies) and marine (34 studies) environments in biodiversity hotspots. Only half of the hotspots have studies on climate change impacts. Biodiversity hotspots are expected to be especially vulnerable to climate change because their endemic species have smaller geographic areas (Sandel et al 2011; Brown et al., 2020).

We need to protect the nature that sustains us. We can do this by reducing our impact on the environment, supporting sustainable development and conserving wild places. It is estimated that one in eight species of plants and animals is at risk of extinction. A projected decrease in habitat suitability for large species like the Asiatic black bear (Ursusthibetanus) is of concern as alternative habitats are outside protected areas, and may lead to human–wildlife conflicts (Farashi and Erfani, 2018). The few positive impacts of climate change were projected as increases in suitable habitat and distribution range for a few endangered plants and mammals (medium confidence) (Banag et al., 2015; Shrestha et al., 2018). Animals benefiting from increased fruit and seed production in Southeast Asian forests during warm El Niño cycles were also projected to increase with climate warming (Corlett, 2011).

Biodiversity is essential for the health of our planet and our well-being. By protecting biodiversity, we are actually protecting our future and upcoming generation. We can all protect biodiversity by supporting many conservation efforts. Some of the efforts are as follows:

- Reduce your carbon footprint by driving less, using less energy, and eating less meat.
- Avoid using pesticides and herbicides in our yard and garden.
- Support sustainable agriculture and forestry practices.
- Reduce your consumption of goods and services that require a lot of resources to produce.
- Get involved in conservation efforts in your community.

A hotspot is a place where there is a high concentration of something. This can be anything from people to businesses to biodiversity.Hotspots can be both positive and negative. For example, a biodiversity hotspot is a positive hotspot because it is home to a large variety of plants and animals. However, a population hotspot can be a negative hotspot because it can create challenges such as overcrowding and pollution.

Some of the examples of different types of hotspots -

- Biodiversity hotspots: Areas with a high concentration of plant and animal species, many of which are endemic. Eg. the Amazon rainforest, the Congo rainforest, and the Coral Triangle are all biodiversity hotspots.

- Population hotspots: Areas with a high concentration of people. Eg. Tokyo, Delhi, and São Paulo are all population hotspots.
- Business hotspots: Areas with a high concentration of businesses. Eg. New York City, London, and Tokyo are all business hotspots.

A biodiversity hotspot is a bio-geographic region with significant levels of biodiversity that is threatened with destruction. The term was first coined by Norman Myers in 1988.

To qualify as a biodiversity hotspot, a region must meet two strict criteria:

1.It must have at least 1,500 vascular plants as endemics — which is to say, it must have a high percentage of plant life found nowhere else on the planet. A hotspot, in other words, is irreplaceable.

2.It must have 30% or less of its original natural vegetation. In other words, it must be threatened.

There are currently 35 biodiversity hotspots in the world, covering just 2.5% of the Earth's land surface but containing 44% of its plant species and 35% of its vertebrate animals. Terrestrial and freshwater hotspots have been warming less over the last 50 years than non-hotspot areas, whereas marine hotspots have been warming more (Kocsis et al., 2021).

The hotspots are concentrated in the tropics and subtropics, and many of them are located in developing countries. They are also home to some of the world's poorest people, who depend on these ecosystems for their livelihoods. The main threats to biodiversity hotspots are deforestation, agriculture, mining, and climate change. These activities are destroying habitats, killing species, and disrupting ecosystems.

There are a number of things that can be done to conserve biodiversity hotspots, including:

- Protecting existing protected areas
- Creating new protected areas
- Reducing deforestation and other forms of habitat destruction
- Promoting sustainable agriculture and development
- Reducing our impact on the climate

By taking these steps, we can help to protect the biodiversity hotspots that are so important to our planet. It is important to be aware of hotspots so that we can understand the challenges and opportunities that they present. We can then work to address the challenges and maximize the opportunities.

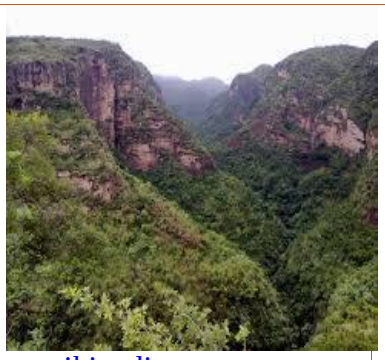
Hotspots of Madhya Pradesh

Madhya Pradesh is a state in central India with a rich and diverse natural heritage. It is home to a variety of ecosystems, including forests, grasslands, wetlands, and mountains. This diversity is reflected in the state's wildlife, which includes tigers, leopards, elephants, sloth bears, and a wide variety of birds and reptiles. It is one of the most biodiverse states in India, with a wide variety of flora and fauna. The state is home to over 10,000 plant species, 1,200 animal species, and over 500 bird species. The state's biodiversity is due to its varied topography, which includes the Vindhya Mountains, the Satpura Range, the Malwa Plateau and the Chambal Valley. These different habitats support a wide variety of plants and animals.

Some of the most popular hotspots for nature tourism in Madhya Pradesh include :

S.No.	Images	Location	Description
1.	 en.wikipedia.org	Kanha National Park, Madhya Pradesh	Kanha is one of the best places in the world to see tigers. It is also home to a variety of other wildlife, including leopards, sloth bears, gaur, and sambar deer.

2.	 en.wikivoyage.org	Bandhavgarh National Park, Madhya Pradesh	Bandhavgarh is another popular tiger park in Madhya Pradesh. It is also known for its ancient forts and temples
3.	 en.wikipedia.org	Panna National Park, Madhya Pradesh	Panna is a former tiger sanctuary that was reintroduced to tigers in 2018. It is also home to a variety of other wildlife, including leopards, sloth bears, and dhole.
4.	 www.tourmyindia.com	Satpura National Park, Madhya Pradesh	Satpura is a large national park that is home to a variety of wildlife, including tigers, leopards, dhole, and sloth bears. It is also known for its beautiful waterfalls and canyons.

5.	 en.wikipedia.org	Pachmarhi Hill Station, Madhya Pradesh	Pachmarhi is a hill station in Madhya Pradesh that is known for its beautiful scenery and pleasant climate. It is also home to a variety of wildlife, including leopards, sloth bears, and wild boars.
6.	 iasbaba.com	Bhoj Wetland, Madhya Pradesh	Bhoj Wetland is a large wetland in Bhopal that is home to a variety of birds and reptiles. It is also a popular spot for boating and other water activities.
7.	 en.wikipedia.org	Van Vihar National Park, Madhya Pradesh	Van Vihar is a national park in Bhopal that is home to a variety of wildlife, including leopards, sloth bears, and deer. It is also a popular spot for birdwatching.

8.	 www.penchnationalpark.com	Pench National Park, Madhya Pradesh	Pench is a national park in southern Madhya Pradesh that is known for its tiger population. It is also home to a variety of other wildlife, including leopards, sloth bears, and wild dogs.
9.	 www.tourmyindia.com	Bhedaghat, Madhya Pradesh	Bhedaghat is a town in Jabalpur that is known for its marble rocks and Dhuandhar Falls. It is also a popular spot for boat rides and other water activities.
10.	 en.wikipedia.org	Amarkantak, Madhya Pradesh	Amarkantak is a pilgrimage site in the Chhindwara district of Madhya Pradesh. It is also the source of the Narmada River.

These are just a few of the many hotspots for nature tourism in Madhya Pradesh. With its diverse landscapes and rich wildlife, the state has something to offer everyone.

Conclusion

Madhya Pradesh is home to a wide variety of ecosystems and a rich diversity of plant and animal species. It is home to two biodiversity hotspots: the Satpura and Vindhyachal Ranges. The state's biodiversity is important for a number of reasons, including ecosystem services, tourism, and cultural heritage. Madhya Pradesh's biodiversity is under threat from habitat loss, poaching, and climate change. It is important to conserve Madhya Pradesh's biodiversity for future generations.We can all do our part to conserve Madhya Pradesh's biodiversity by supporting sustainable practices and reducing our environmental impact.

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Microbial synthesis of Exopolysaccharides and its applications

K. Gautam*[1], R. Sankar[1], J. Prasanna[2], S. Mahendran[3], P. Maheswari[3], M. Nivetha[3]

[1]P.G. & Research Department of Microbiology,
Asan Memorial College of Arts & Science, Chennai-600 100,
Tamil Nadu, India

[2]Department of Microbiology,
Vivekanandha College of Arts & Sciences for Women, Elayampalayam,
Namakkal-637 205, Tamil Nadu, India

[3]Department of Microbiology, Ayya Nadar Janaki Ammal College
(Autonomous), Sivakasi-626 124, Tamil Nadu, India

Introduction

Bacteria produce diverse biopolymers with varied chemical properties through utilization of simple to complex substrates.A wide variety of structural, useful and profitable polysaccharides are produced by microbial cells(both eukaryotic and prokaryotic), with respect to cellular location, biopolymers could either be intracellular or extracellular. The intracellular biopolymers are few and have very limited use; however, the range of the extracellular biopolymers are vast and may be grouped into four major classes; polysaccharides, inorganic polyanhydrides (such as polyphosphates), polyesters and polyamides (Rehm, 2010), and have been collectively termed extracellular polymeric substances, slime and microcapsular polysaccharides.

Polysaccharide components of the extracellular biopolymers are the most abundant and their location relative to the cell, again, forms the basis for their classification. At the cell wall, they serve structural and protective purposes and are found as constituents in teichoic acids. Outside the cell, they may take the form of a covalently bound cohesive layer; a morphologic entity termed capsule or completely excreted into the environment as slime.

There are four general mechanism known for the production of the carbohydrate polymer in bacteria i) Wzx/Wzy-dependent pathway, ii) the ATP-binding cassette (ABC) transporter dependent pathway, iii) the synthase-dependent pathway and iv) the extracellular synthesis by use of a single sucrase protein.

Morphologic and Functional Properties of Bacterial Exopolysaccharide

The history of bacterial exopolysaccharides began during the mid-19th century with the discovery of an exopolysaccharide in wine, which would later be known as dextran and the prokaryote responsible for the production was identified as *Leuconostoc mesenteriodes*, Later other exoplysaccharides discovered includes cellulose, alginate and xanthan. Advances in science led to the use of bacteriolytic enzymes and radioisotope labeling of precursors for biosynthetic studies thus, some details about the metabolic pathways for biopolymer formation were elucidated (Pindar and Bucke, 1975). An instance is in the elucidation of capsular polysaccharide from *Klebsiella* K15 using 1D and 2D ^{1}H and ^{13}C NMR spectroscopy of the alditol (an oligosaccharide) obtained by depolymerisation of the polysaccharide with a viral-borne endoglycanase.

Bacterial Capsule

Many bacterial cells secrete some extracellular material in the form of a capsule and is attached tightly to the bacterium and has definite boundaries. A capsular layer of extracellular polysaccharide material can enclose many bacteria into a biofilm and serves many functions. Some bacteria are taxonomically grouped based on their capsular polysaccharides and this is exemplified in *E. coli* were *O*-antigen; cell wall derived, *H*-antigen; flagella derived, and K-antigen; capsule derived forms antigenic classification of the organism. The *K*-antigen is further divided into L, B and A groups(Bugg and Brandish,1994).Capsules provide multiple functions to the bacterial cells, which include, as adhesion receptors during colonization of tissues. Moreover, the multiple variations in structure confer resistance to various phages and vertebrate complement.

Exopolysaccharides

Bacterial polysaccharides synthesized and secreted into the external environment or synthesized extracellularly by cell wall-anchored enzymes may be referred to as exopolysaccharides.Microbial polysaccharides are produced in two forms, capsular polysaccharide (CPS) and exopolysaccharide (EPS). EPSs of microbial origin are ubiquitous in nature, have unique properties, and can be isolated from the bacteria in fresh water, marine environment, extreme conditions, and soil ecosystem. Exopolysaccharides are comprised of repeated units of sugar moieties, attached to a carrier lipid, and can be associated with proteins, lipids, organic and inorganic compounds, metal ions, and DNA.It's function depends on structural units and ecological niches of the host microorganisms and have various commercial application ranging from

pharmaceutical to food-processing, extended to detoxification, bioremediation, paints, biotechnology, and petrochemicals. Based on monomeric composition and as such; homopolysaccharides and heteropolysaccharides are the two groups recognized. Homopolysaccharides contain only one type of monosaccharide while heteropolysaccharides is composed of repeating units, varying in size from disaccharides to heptasaccharides.

Exopolysaccharide categorization are complex and it further make distinctions between groups and this is seen in homopolysaccharides been further clustered into four groups thus; α-D-glucans, β-D-glucans, fructans and polygalactan; this grouping is based on linkage bonds and nature of monomeric units. On the other hand, the composition of heteropolysaccharides includes the repeating units of D-glucose, D-galactose, L-rhamnose and, in a some instance, *N*-acetylglucosamine (GlcNAc), *N*-acetylgalactosamine (GalNAc) or glucuronic acid (GlcA). Non-carbohydrate substituent such as phosphate, acetyl and glycerol are sometimes present. Bonds between monomeric units at the backbone of the polymers are 1,4-β- or 1,3-β-linkages and 1,2-α- or 1,6-α-linkages. The differences between homopolysaccharide and heteropolysaccharide are not only reflected in the chemical nature and linkage bonds but in synthetic enzymes and site of synthesis.Structural exopolysaccharides includes neutral polysaccharides as they serve architectural purposes in the matrix facilitating water retention and cell protection. Surface active exopolysaccharides includes molecules with amphiphilic behavior; they have varied chemical structures and surface properties and may be involved in biofilm formation and/or sometimes possess antibacterial or antifungal activities. Sorptive exopolysaccharides are composed of charged polymers, whose function is sorption to other charged molecules involved in cell-surface interactions(Esko, 1999).

Exopolysaccharides in bacterial biofilm

In nature, bacteria exists in colonies accumulating at interfaces to form poly-bacterial aggregates such as mat, flocs, sludge.The use of term biofilms to mean microbial aggregates that accumulate at a solid–liquid interface and are encased in a matrix of highly hydrated extracellular biopolymers. Although this description does not take into account groups of free floating microbial aggregates (flocs).

Biofilms have been metaphorically dubbed “city of microbes” (Daegelen *et al*.,2009), and the extracellular biopolymers, in which exopolysaccharide predominates, as the “house of the biofilm cells” (Schembri *et al*., 2004).

Bacterial Exopolysaccharides Antigen

Bacterial exopolysaccharides are limited to all forms of polysaccharides synthesized and secreted into cellular external environment which may remain loosely attached to the surface (capsule) or completely detached. Polysaccharide capsular constituents (polysaccharides and/or glycol-conjugates of protein and lipids) represent major surface antigens for slimy bacteria and their role in pathogenicity has been extensively investigated. However, due to the great diversity shown by the exopolysaccharides with respect to monomeric units, linkages, and unique structures, varied immunogenic responses are elicited and these antigenic properties are inclusive in serologic grouping of bacteria(Marvasi *et al*.,2010). This is seen in *Enterobacteriaceae* where over 80 different serotypes of *E. coli* have been identified based on capsular polysaccharide antigen (*K* antigens) (Schembri *et al*.,2004).

Capsular polysaccharide antigenicity cuts across Gram status divide; this is reflected in *N. meningitidis*, *E. coli* and *Salmonella typhi* (Gram-negatives) and *Staphylococcus* spp. and *Streptococcus* spp. (Gram-positive). Capsular polysaccharide based bacterial serotyping is predicated on reactivity of specific antibodies, often generated in animals, using reference strains of particular species, with the culpable bacteria. The polysaccharides structural diversity leads to various kinds of antibody reactivities as reflected in the large numbers of serotypes found within bacteria of the same species. Epidemiologically, bacterial serotyping has been of great importance because it is a simple and rapid procedure and comes handy in disease outbreaks. In epidemics, it is important to monitor the spread of causative agents as certain diseases caused by some bacterial species may be limited to a few serotypes. Structural elucidation of bacterial surface polysaccharides and advances in immunology has led to the development of capsular polysaccharide based vaccines(Flemming *et al*., 2007), which has been largely successful in preventing infectious disease.Some clinically important bacteria with its pathogenic serogrouping sequel to their capsule

Microbial biosynthesis of exopolysaccharide

Biosynthesis of EPSs in bacteria occurs in intra- and extracellular ways. There are four general mechanisms for EPS biosynthesis in bacterial cells, which are the Wzx/Wzy-dependent pathway, the ABC transporter-dependent pathway, the synthase-dependent pathway, and extracellular biosynthesis by sucrase protein (Angelin *et al*.,2020; Rana and Upadhyay,2020).

WZX/WZY-Dependent pathway

The Wzx/Wzy-dependent pathway consists of three stages: (i) synthesis of nucleotide sugars, (ii) assembly of repeat units, and (iii) polymerization and export. In the first stage, the sugar residues are actively transported into cells and transformed into various monomeric units, which are transferred toward and linked to an undecaprenyl phosphate (Und-P) anchor (C55 lipid carrier) at the inner membrane(De Vuyst*et al*.,2010; Rehm,2010). In the second stage, glycosyltransferases (GTs) link more sugar units to produce repeating units, which translocate across the cytoplasmic membrane by Wzx flippase. For the last stage, the translocated oligosaccharide units undergo various enzyme modifications, such as methylation and acetylation, and are polymerized to polysaccharides by the Wzy protein(Islam and Lam,2014). The polysaccharides assembled via the Wzx/Wzy-dependent pathway are HePs containing highly diverse sugar units. The assembled polysaccharides are released to the cell surface by ABC transporters. In probiotic bacteria, the HePs are gellan, xanthan, and kefiran, produced by the Wzx/Wzy-dependent pathway(Angelin and Kavitha.,2020).

ABC Transporter-Dependent Pathway

In the ABC transporter-dependent pathway, such as that employed in the synthesis of *Myxococcus xanthus* EPS, the active sugar units are transported to an Und-P molecule at the inner membrane to form an Und-PP-sugar molecule. The full-length polysaccharides are synthesized by specific GTs located at the cytoplasmic side's inner membrane and then translocated across the inner membrane by a tripartite efflux pump complex in the inner membrane. This pathway is mainly involved in synthesizing capsular polysaccharides(Perez-Burgos *et al*.,2020, Huszczynski*et al*.,2020).

Synthase-dependent pathway

The polymer products based on the synthase-dependent pathway are HoPs made from a single type of sugar unit, such as bacterial ALG and cellulose. In the synthase-dependent pathway, the assembly of UDP-glucose units occurs by a membrane-embedded synthase/inner membrane transporter bacterial cellulose synthesis (bcs) A(Krasteva *et al*.,2017). Bacterial cellulose synthesis operons are highly variable and species-dependent.

Extracellular biosynthesis by sucrase protein

In the extracellular biosynthesis pathway, sucrose is transformed by extracellular sucrase enzymes into monomeric units outside the cellular outer membrane. The GTs polymerize the monosaccharide units into glucan

(dextran) and fructan (levan) with different branches(Lynch *et al*.,2018). In probiotic bacterial cells, glucan sucrases are categorized as alternan sucrases (alternan), dextran sucrases (dextran), mutan sucrases (mutan), and reuteran sucrases (reuteran). In contrast, fructan sucrases are separated into levan sucrases (levan) and inulin sucrases (inulin). EPSs synthesized in probiotic bacteria by the extracellular biosynthesis pathway are HoPs. For example, the glucose monomer is the component of dextran, mutan, alternan, reuteran, and curdlan, while levan and polygalactans are made from fructose and galactose, respectively(Angelin and Kavitha,2020). The synthesized EPSs are released to the extracellular environment.

Extraction and purification of exopolysaccharide from lactic acid bacterium

1. Lactic acid bacterium was cultured at 37^{o}C for 18-24 hours in MRS modified medium supplemented with 10% glucose.
2. After centrifugation (8,000 ×g for 20 min at 4^{o}C) of culture, the supernatant was collected and added with a final concentration of 14% trichloroacetic acid to denature the protein content.
3. The culture was further left for homogenization in a shaker (90 rpm) for 30-40 min followed by centrifugation at 8,000 ×g for 20 min at 4^{o}C.
4. The supernatant was then added to cold absolute ethanol (two-fold volume of supernatant) at 4^{o}C for 24 hours, followed by centrifugation at 8000 ×g at 4^{o}C for 20 min
5. These steps resulted in the isolation of crude precipitate.
6. Finally, the precipitate was dissolved in deionized water and dialyzed using Spectra/Por molecularporous tubular dialysis membrane for 24~48 hours.
7. The precipitate was then lyophilized in an freeze dryer.
8. The freeze-dried lyophilized powder of lactic acid bacterium was considered to be purified exopolysaccharides.
9. The purified exopolysaccharide was stored at -80^{o}C for further analysis.

Applications of Bacterial Exopolysaccharides

The discoveries of numerous types of exopolysaccharides have been documented. However, only a few have been shown to have industrial and medical relevance with significant commercial value, particularly with regard to their use as biomaterials. The limitation of the applications of some of these

bacterial polysaccharides has been largely due to cost of production relative to their commercial value; however recover the problem using cheaper substrates, improving product yield by optimizing fermentation conditions, or developing higher yielding strains via mutagenesis, and/or genetic and metabolic manipulations, and optimizing downstream processing. Advances in the application of bacterial exopolysaccharides in medicine and biotechnology have seen uses to include bacterial alginate in cell microencapsulation, such as microsphere vectors for drug delivery, making dental impressions, as an active ingredient in absorbent dressings, and anti-reflux therapies (Flemming and Wingender, 2002). Likewise, dextran, produced by *Leuconostoc mesenteriodes*, have been used to prepare one of the most effective plasma substitutes for application in shock and the loss of blood (Silver *et al*.,1998).

Glycosaminoglycan heparins, used in the prevention and treatment of thromboembolic disorders, have been associated with inefficacy in antithrombin deficient patients with side effects as bleeding and thrombocytopenia. Thus, sulphated forms of alginate have been thought to serve as an alternative with enhanced activity. Other therapeutic activities attributed to sulphated forms of alginate includes; anticoagulant, antithrombotic, anti-atherosclerotic, anti-angiogenesis, anti-metastatic and anti-inflammatory(DeAngelis and White, 2002). Xanthan gum produced by *Xanthomonas campestris* has broad industrial application. Industrial applications are broad and include areas such as in foods, toiletries, oil recovery, cosmetics and as water-based paints among other. Superior rheological properties shown by xanthan gum allow it to be used as rheological control agent in aqueous systems and as stabilizer for emulsions and suspensions.

In the agriculture sector, the flow ability in fungicides, herbicides, and insecticides has been improved by the addition of xanthan to uniformly suspend solid component in formulations. Furthermore, ability to disperse and hydrate rapidly as well as non-pollution and good color yield status attributed to xanthan have ensured its use in jet injection printing. Thermo-set coatings have included xanthan gum as it is very environmentally friendly. Similarly, the petroleum industry uses xanthan gum in oil drilling, fracturing and pipeline cleaning (Roberts, 1996) and due to its excellent compatibility with salt and resistance to thermal degradation, it is also useful as an additive in drilling fluids.

Microbial glycosaminoglycans (GAG) are capsular polysaccharides produced by *Escherichia coli* K5, *E. coli* K4, and *Pasteurella multocida*. GAGs are structurally linear polysaccharides, composed of repeating disaccharide units derived from amino sugars (glucosamine or galactosamine), hyaluronan, chondroitin and heparan sulphate with uronic acid as the other component of the disaccharide repeat. The biological functions of GAGs includes molecular camouflage for pathogenic bacteria but these polysaccharides have similar backbone structure as the commercial heparin and have been synthesized by *Escherichia coli* K5 in the non sulphated forms and in the sulphated forms in *E. coli* K4. *P. multocida*, similarly, produces heparan sulphate with molecular weights between 200 and 300 kDa(Rosalam and England, 2006), which is higher than those of *E. coli* K5 and *E. coli* K4. GAGs are shed into the environment (fermentation medium) hence; their recovery and modification into biologically active GAGs, such as heparin or heparosan sulphate will serve as an important substitute for animal derived GAGs(Yoshida and Tanner, 1993). Important medicinal products may be obtained from GAG producing bacteria as is the situation with Group C Streptococcus (GCS). GCS serves as an important commercial source of hyaluronan polysaccharide for surgical, ophthalmic and viscoelastic applications.

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Azolla – A Feed additive

Muniasamy[1], S. Gowtham[1], P. Ponmanickam[2]
[1]Department of Microbiology, [2] Department of Zoology
Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi – 626 124.
E-mail: *ayya.mari@gmail.com*

Abstract

In tropical countries, there are several fast growing plants, which can synthesize proteins from atmospheric nitrogen through nitrogen fixation. *Azolla* is a fern that float on water, fix atmospheric nitrogen using the commensal *Azollae anabenae*. Azolla can be cultivated in a large scale with minimum effortand it is used in agriculture as fertilizer, weed controlling agent in paddy fields, fodder for small, companion animals, supplemented food for poultry, piggery and more.

Key words: Azolla, Fodder, Supplemented feed.

1.0 Introduction

Plants are the autotrophic organisms that can synthesize the carbohydrates, proteins other essential biomolecules for their survival and reproduction. In this respect there are different legumes, like soya, peanuts, whereas the Azolla is free living water fern that can fixes nitrogen from atmosphere. In rice cultivation, Azolla is used as green manure as a substitute for inorganic nitrogen fertilizers. The term *Azolla* was first coined by Lamarck in 1783. The genus *Azolla* belongs division Pteridophyta, class Polypodiopsida order Salviniales. It also belongs to the family Salviniaceae consisted of two subgenera six living species (Lumpkin, 1984). The subgenus *EuAzolla*, characterized by three megaspore floats septate glochidia, included four species: *A. filiculoides* Lain., *A. caroliniana* WiUd., *A. microphylla* Kaulf., *A. Mexicana* Presl. The subgenus *Rhizosperma,* characterized by nine megaspore floats, included two species: *A. pinnata* R. Br., with simple glochidia, *A. nilotica* Decne., with no glochidia.

2.0Distribution

Azolla generally grows in freshwater (Fig.1), tropical, subtropical, warm-temperate regions throughout the world. *Azolla caroliniana* is found in eastern North America the Caribbean, *A.filiculoides* in the Americas from

southern South America through western North America to Alaska, *A. microphylla* in tropical subtropical America, *A. mexicana* in the Americas from northern South America through western North America, *A.nilotica* in East Africa from Sudan to Mozambique, *A.pinnata* in most of Asia the coast of tropical Africa (Watanabe, 1982; Wagner 1997).

3.0 Morphology

Three *Azolla* sp. *i.e, A. caroliniana, A. microphylla, A. pinnata* are commonly found all over the Indian subcontinent. The macrophyte of *Azolla* is called frond which ranges from 1 cm to 2.5 cm in length in species such as *A. pinnata* 15 cm or more in the largest species like *A. nilotica* (Raja *et al*., 2012). It has a main rhizome which branches into secondary rhizomes, all of which bear small leaves alternately arranged. Numerous unbranched, adventitious roots hang down into the water from nodes on the ventral surfaces of the rhizomes. The roots absorb nutrients directly from the water in shallow water they may touch the soil, deriving nutrients from it. Each leaf consists of two lobes: an aerial dorsal lobe, which is chlorophyllous, a partially submerged ventral lobe, which is colourless cup-shaped provides buoyancy. Each dorsal lobe contains a leaf cavity which houses the symbiotic *Anabaena azollae* (Peters, 1977). The interior surface of each leaf cavity is lined with an envelope (Peters, 1975) covered by a mucilaginous layer of unknown composition which is embedded with filaments of *A. azollae* permeated by multicellular transfer hairs (Roy *et al*., 2016).

It grows in association with the blue-green Anabaena *azollae*, that living in the cavity of *Azolla* leaf, can fix high amount of atmospheric nitrogen due to presence of symbiotic algae in the leaves (Becking, 1979). It is considered to be the most promising because of the ease of cultivation, high productivity good nutritive value (Singh and Subudhi, 1978; Prabina and Kumar, 2010). It produces maximum biomass in a relatively shorter period of time (Brouwer *et al.,* 2017). It is commonly called mosquito fern, duckweed fern, or water velvet. The water fern Azolla (*Azolla pinnata*) is an unconventional feed ingredient. The endosymbiont provides sufficient nitrogen for both itself its hosts (Peters., 1975).

4.0 Applications of *Azolla*

As Biofertiilizer

Azolla-Anabaena complex has great potential as a biofertilizer, because it can assimilate atmospheric nitrogen efficiently. It is a common biofertilizer in rice crop. Thus, Azolla appears to be a potential source of nutrients has a

considerably high feeding value. The higher crude protein content (above 20%) presence of essential amino acids (high lysine content), vitamin A precursor beta-carotene, vitamin B12 minerals like iron, calcium, phosphorous, potassium magnesium made Azolla a useful feed supplement for livestock, poultry fish. It is also found to contain probiotics biopolymers (Pillai *et al*., 2002). The dietary *Azolla* supplementation shows to have a positive effect on growth performance of fish reduce the cost of feeding on fish meal fish oil diet (Mosha., 2018). Also, it can be utilized as a feed for animals/birds (Shukla., 2018), food for humans, water purifier, green fertilizer or vermicompost (Aroraand Kaur, 2019) biogas (Sathammaipriya*et al*.,2018),Biolarvicide, to improve soil microbial diversity (Ravi, 2018).Other constituents in *Azolla* are minerals, chlorophyll, carotinoids, aminoacids, vitamins etc. It is also a potential source of nitrogen is a potential feed ingredient for livestock (Lumpkin, 1984; Pannerker, 1988)

The greens (green plants) have long been recognized as the cheapest most abundant potential source of proteins because of their ability to synthesize aminoacids from a wide range of virtually unlimited readily available primary materials (Fasuyi and Aletor, 2005). The use of Azolla as a feed resource for fish, swine poultry had been tested with favourable results (Castillo *et al*., 1981; Alcantara and Querubin, 1985). Utilization of aquatic plants weeds having high food value as feed ingredients has taken a new dimension in producing the much required animal protein at low cost. Aquatic weeds also have the added advantage of being cultivated in association with farmed fish species using the same water resources /or farm effluent (Edwards *et al.,* 1992; Gavina, 1994).

As Feed

The cost of production of aqua feed can be reduced using this ecofriendly sustainable resource. The use of azolla as a fish feed ingredient is well documented, but literature on its dietary use in seed rearing is scanty. *Azolla* has been traditionally used as a green manure an animal feed in Vietnam, China (Lumpkin Plucknett 1980).

Incorporation of azolla as an alternative protein ingredient in poultry ration could make poultry production economical. In case of commercial broiler chickens, *Azolla* can be efficiently used as a feed ingredient in the form of sun-dried ground*Azolla* meal. *Azolla* meal can partially replace the dietary protein sources up to 5-10% without any adverse effect on the health performance of the birds. Inclusion of *Azolla* in the poultry diet helps in the economization of production cost; thus, increasing the net profit. Similar

findings have been observed in the case of quails with optimum displacement level of *Azolla* restricted to 5%. The high carotene content of *Azolla* is responsible for its immune-potentiating effect in poultry birds. In non-ruminants, essential amino acids, linoleic acid, vitamin A, folic acid, vitamin B6, vitamin B12, vitamin C, vitamin E, zinc, copper, iron, selenium affect one or more indices of immunity. Use of *Azolla* as an antibacterial, antioxidant agent in complementary alternate medicine had been recommended due to its high phenolic, flavonoid content. Antioxidant activity of *Azolla* had been successfully demonstrated in Swiss albino mice (Chichilichi*et al.,* 2015).

Azolla has a higher crude protein content (ranging from 19 to 30 per cent) than most green forage crops aquaticmacrophytes rather favorable essential amino acid (EAA) composition for animal nutrition (rich in lysine), it has also attracted the attention of livestock, poultry fish farmers (Cagauan andPullin, 1991).It is used as a fish feed ingredient of an alternative protein source, is well documentedin black tiger shrimp *Penaeus monodan* (Sudaaryano, 2006), in carps (Maity and Patra, 2008) Nile tilapia (Youssouf, 2012) it can convert its raw protein into best edible protein. Thus the cost of production of aqua feed can be reduced using an eco-friendly sustainable resource (Datta, 2011). Cohen *et al*., (2002) reported that the 3-Deoxyanthocyanins are the only known flavonoids of Azolla. The crude extract of *A. pinnata* contains various antioxidant like phyto-constituents such as, tannins, phenolic contents flavonoids (Radhakrishnan *et al*., 2014).

With the rapid growth in the aquaculture sector in the recent years, the demand for quality fish feed is continuously increasing. Providing quality fish feed became a prime aim of every aqua culturist. Though fish feed devours around 50% of the production cost, yet it plays the pivotal role in the production yield outcome (Mzengereza *et al*., 2014). With the increase in plea, the price of feed increased simultaneously. One main reason for the rise in the cost price of fish feed is due to the rise in demand of fish meal which remains the core of the protein supply of the feed.

The mandate for fish meal is not just increasing but the supply of it is also dwindling with stagnating marine catches alternative use of it for livestock, human consumption (Fasakin *et al*., 1999). So, the quest for possible alternative protein sources to replace (complete/partial) fish meal in the feed became paramount (Magouz *et al*., 2008). As animal protein sources are mostly expensive not easily available, plant sources are considered to be one possible alternative that can be used in fish feed without compromising the nutritional quality of the feed (El-Sayed, 1999; Francis *et al*.,2012). Moreover, use of

cheaper locally available plant sources to substitute the expensive fish meals would mean reduction in the production cost thereby enhance the profit (Osman *et al*., 1996, Munguti *et al*., 2006).

The prime consideration of selecting a fish feed ingredients formulation of the feed is the digestibility, palatability, acceptability by the fish, availability cost of the ingredients (Rodriguez *et al*., 1996; De Silva Anderson 1995). Incorporation of plant sources in fish feed generally has its limitation due to low protein content, presences of anti-nutritional factors such as alkaloids, glycosides, oxalic acids, phytates, protease inhibitors, haematoglutinin, saponegin, momosine, cyanoglycosides etc. their imbalances in some essential amino acids, fatty acids micronutrients (Abowei *et al*.,2011).

The potential of the feedstuffs to be used in fish diets can be established based on their proximate chemical composition (Mzengereza *et al*., 2014). Efforts are made to remove the anti-nutritional factors in the plant sources. Before incorporation into diets to reduce anti-nutritional factors the leaf meals were soaked in water, dried, ground to a small particle size (Lochmann *et al*.,2011). In fish feed many research has been done on use of soybean, rapeseed (canola) meal, cottonseed meal, sunflower seed meal, wheat corn gluten, peanut meal, moringa leaves meals etc. (Makkar and Becker, 1996; Francis *et al*.,2012; Egwui *et al*.,2013; Mondal and Payra, 2015, Dorothy *et al*., 2018).

As immune boosters

Along with enzyme supplementation in the isonitrogenous diet with Azolla showed better immune response in broiler chickens, supplying 10% of protein requirement ((Biswal Chichilichi, 2015). Azolla has beneficial effects on the digestive enzymes, intestinal morphometry, immune functions, growth performance of GIFT (Magouz *et al.*, 2020).

4.0 Conclusion

Azolla is a good feed additive for all kind of animals because of its high content of proteins and low carbohydrate as well lipid content. It can be easily incorporated in the ration feeds of broilers, fish and others veterinary animals. Moreover, the use of azolla does not affect the quality of food/ fodder and enhances the growth of individual animal as well as improves its vital functions.

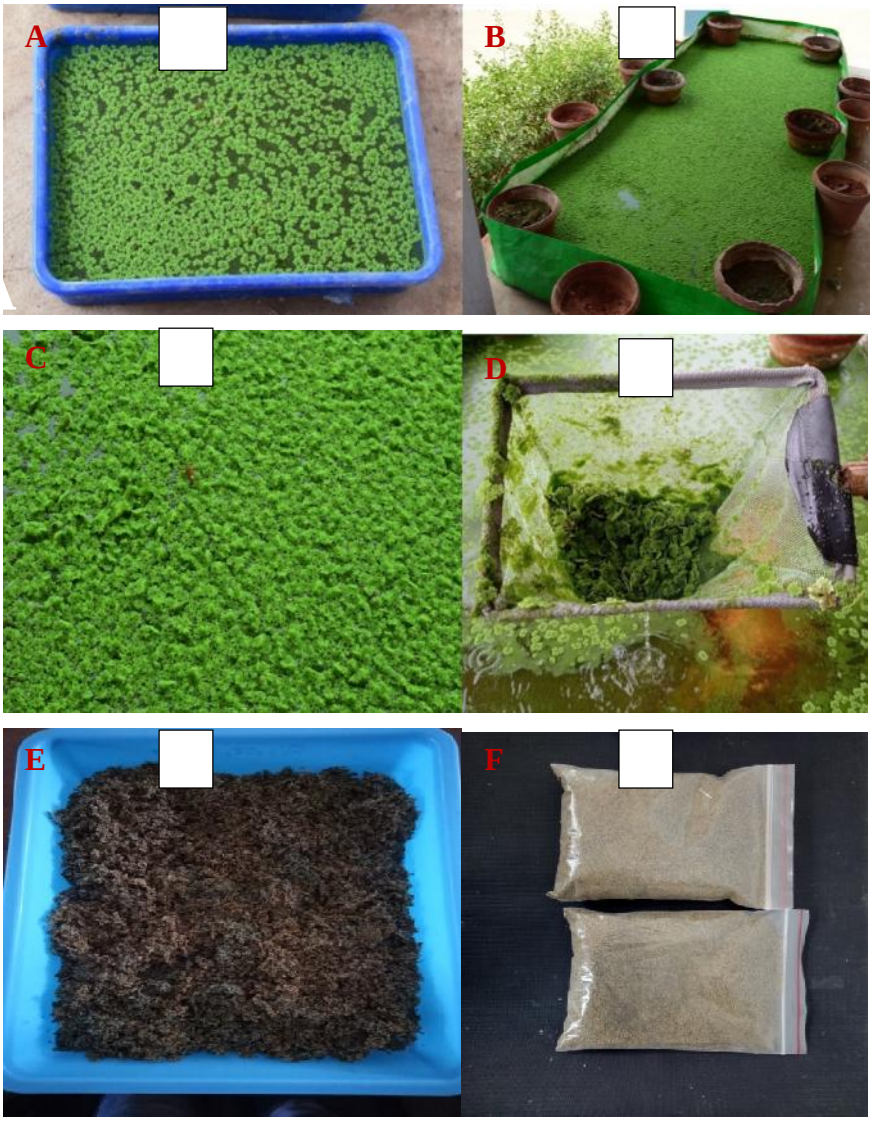

Fig. 1. Azolla cultivation

A. Small tray cultivation, B. Medium sized bed cultivation, C. Matured Azolla, D. Harvested Azolla, E. Shade dried azolla, F. Powdered Azolla.

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Fungi as Microbial fuel cell

P. Maheswari[1] and S. Ashok[2]
Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi, Tamil Nadu
[1]Assistant Professor, Department of Microbiology,
E-mail: *mahes02@gmail.com*
[2]II M.Sc., Microbiology, E-mail: *ashokgkm2001@gmail.com*

Introduction

Recently, microbial fuel cells (MFCs), a method of turning organic matter into power, have drawn a lot of attention. In this work, a microbial fuel cell based on compost that produces bioelectricity through the biodegradation of organic materials is created. As organic waste, grass clippings were employed together with leaf mold, rice bran, oil cake (produced by mustard plants), and chicken droppings (chicken waste). The impact of various membrane types, combining fly ash, and various electrode materials was examined along with the electric characteristics of the MFC under anaerobic fermentation conditions (Tan, e*t al.*, 2021.).

Microbial fuel cells work by using microorganisms to break down the organic material in a substrate while simultaneously producing power. During an incomplete respiration process, the electrons produced by metabolism are instead donated to the surface of an electrode through (a) direct electron transfer, (b) intermediate metabolites acting as electron shuttles through a redox pair, and (c) micro-cilia (wires) that directly transport the electrons. These electrons are used by the cathode to reduce the oxidant (for example, oxygen) and flow through an external circuit. Protons produced by fuel oxidation are transported from the anode to the cathode through a compartment and burned in the cathode as part of this process (Trapero, *et.al.*,2017).

Despite the fact that both yeast and bacteria were involved in the pioneering 1911 discovery of the electrical effects associated with organic matter decomposition, fungi-based MFCs received less attention than bacterial ones. The absence of validated fungal electrogens and the lower power output of MFCs operating on single fungus strains in compared to bacteria were factors in the decline in interest in fungal MFCs. However, during the past ten

years, studies which have concentrated on fungi-based MFCs have emerged, and their findings have demonstrated the fungi's electrogenic potential. Recent research suggests that when the redox enzymes found in the fungal cell membrane, such as ferricyanide reductase or lactate dehydrogenase, are involved, the process of electron transfer performed by fungus may be direct, comparable to bacteria (Sayed and Abdelkareem2017)).The use of fungi as cathode and anode catalysts, or as a supporting element in bacterial MFCs, will be discussed in this article's assessment of the state-of-the-art in the field of fungal-based MFCs.

Mechanisms of microbial fuel cells:

Organic matter is oxidized by microorganisms, generating electrons that go through a number of respiratory enzymes in the cell to produce ATP, the cell's energy currency.The anodic compartment of microbial fuel cells (MFCs) is normally kept in an anaerobic atmosphere and operated in a closed-system mode(Slate *et al.,* 2019).MFCs are an emerging, non-chemical alternative technology that converts organic substrates into electricity using living microorganisms. Additionally, MFC demonstrates additional benefits such minimal sludge generation, the use of a variety of substrates, minimal power usage, and optimal temperature performance.MFCs are an emerging, non-chemical alternative technology that converts organic substrates into electricity using living microorganisms. Additionally, MFC demonstrates additional benefits such minimal sludge generation, the use of a variety of substrates, minimal power usage, and optimal temperature performance(Sayed*et al.,* 2012*;* Schaetzle *et al.,* 2008). A terminal electron acceptor that reduces receives the liberated electrons after that.TEAs like oxygen, nitrate, and sulfate, among others, easily diffuse into the cell where they take electrons to produce products that can also disperse out of the cell. Nevertheless, it is now understood that some bacteria can exogenously (i.e., outside the cell) transfer electrons to a TEA, such as metal oxides like iron oxide. Exoelectrogens, a type of bacteria, can be used in MFC to generate energy under this situation.

Components of Microbial Fuel Cells:

The functional components of MFCs that are crucial to the microbial mediated electrochemical system are electrodes and microorganisms. A proton exchange membrane (PEM), which also serves as a membrane separator,

separates the two chambers that make up a conventional MFC.The MFC is split into two different anodic and cathodic regions by this membrane separator. A bacterium called an exoelectrogen has the capacity to transport electrons extracellularly. Exoelectrogens function as biocatalysts for the oxidation of organic material, which releases electrons that are transmitted from the anode to the cathode and produce electricity, while PEM delivers protons to the cathode.To complete the bioelectrochemical reaction, a water molecule is created at the cathode when a proton and an electron combine.

Anode material:

Anode material is regarded as a crucial factor that influences how well MFCs work. The MFCs' anode should have high surface area, mechanical stability, chemical stability, and electrical stability.The best materials to use as an anode in MFCs with high output power are carbon materials, both conventional and unconventional. Carbon materials have been widely used in MFCs, including conventional ones like carbon paper, cloth, brush, and felt, as well as novel ones like carbon nanotubes (CNTs), carbon nanofibers, and graphene.Non-carbonaceous materials like gold, titanium, and stainless steel have been used in research, but they performed less well than they would have if carbon had been used instead.

Cathode material:

The cathode material, which should have a high redox potential, has a substantial impact on the overall cell voltage. Among the most popular cathodes for MFCs are carbon materials like carbon paper and carbon cloth that have been treated with high active catalysts like Platinum catalyst. The primary obstacles to the use of such a cathode are the expensive cost and scarcity of Platinum. The carbon cloth and/or carbon paper with Platinum considerably reduced the oxygen reduction activation energy and boosted the reaction rate.A variety of non-Platinum-based catalysts, including as carbon nitrogen alloys and metal carbides, were studied as cathodes in MFCs and showed excellent results that gave them a potential to replace Platinum catalyst in the near future.

Separator:

The installation of a separator with a high ionic conductivity and low permeability could increase the performance of the MFC since the anode

operates in anaerobic conditions while the cathode operates in aerobic conditions.Numerous separators, including salt bridges, glass fibers, microfiltration membranes, porous textiles, and coarse-pore filters, have all been thoroughly researched in MFCs.It is important to note that some MFCs performed better even without the divider.

Fungi as Biocatalysts in the Anode of MFCs:

Saccharomyces cerevisiae

S. cerevisiae, or baker's yeast, is a single-celled organism used in the brewing and baking industries. For the study of essential cellular processes like the cell cycle, DNA replication, recombination, cell division, and metabolism, the simple eukaryotic cell *S. cerevisiae* serves as a model system for many eukaryotes, including human cells. *S. cerevisiae* is regarded as a useful biocatalyst in MFC because of its wide range of substrates, simplicity and speed of mass cultivation, no pathogenicity, low cost, and ability to be kept for a long time in the dried condition.Recently, *S. cerevisiae* was utilized in a significant MFC and large-scale extended period in the dry condition.Utilizing complicated substrates in MFCs necessitates the use of microbial consortia where different species can perform a variety of tasks. It is well known that the consortium's fermentative microbes can break down big organic compounds into smaller fermentation byproducts that electrogenic species can utilize.

A microbiological consortium, containing the electrogen*Shewanellaoneidensis* MR-1 and genetically modified S. cerevisiae as a fermenter with glucose as a carbon source, was used in the MFC proposed by (Lin *et al*) *S.cerevisiae* fermenter that has been developed, using glucose as a carbon source.*S. cerevisiae* cells were engineered to use the lactic acid pathway while the ethanol pathway was taken off. *S.cerevisiae* converted glucose into lactic acid as a result, and *S. oneidensis*utilized this lactic acid to produce energy.Achieving the ideal relationship between *S. cerevisiae's* carbon metabolism and *S. oneidensis* extracellular electron transfer boosted the efficiency of such a fermenter-exoelectrogen consortium.*S. cerevisiae* was used because it did not create a biofilm on the anode, allowing the exoelectrogen to completely occupy the anode surface, improving MFC performance. The glucose-fed MFC produced a maximum power density of 123.4 mWm2.

Candida melibiosica

A yeast strain with high phytase activity, *Candida melibiosica* 2491, breaks down the phosphorus compounds found in plant tissues but which are hardly biodegradable. Without the chemical addition of synthetic mediators, Hubenova and Mitov discovered *C. melibiosica's* oxidation-reduction potential, indicating that it may transfer electrons in MFCs.The performance of *C. melibiosica*-based MFCs was also investigated using modified carbon felt as the anode material. With these modified anodes, Ni acted as an electron acceptor or started an adaptive mechanism with a faster rate of electron transfer across the yeast cell membrane, which improved the performance of *C. melibiosica*-based MFCs (Hubenova andMitov2010).The created current, yeast cell development phases, and the rate of substrate assimilation—which was shown by the rate of in vivo produced electrons—have all been found to be correlated. Furthermore, the impact of additional exogenous mediators with different formal potentials (such as bromocresol green (BcG), bromocresol purple, bromothymol blue, bromophenol blue, Congo red, cresol red, eosin, Eriochrome Black T, methyl red (MR), methanyl yellow, methyl orange (MO), murexide, neutral red (NR), and tropaeolin) on the functionality of a *C. melithe* mediator's capacity to accelerate the kinetics of electron transport accounts for the improvement in MFC performance (Babanova *et al.*, 2012).

Arxula adeninivorans

Because the processes of catabolism vary widely between species and under various circumstances in the same species, caution should be taken while choosing a yeast to be the biocatalyst in an MFC. *Arxula adeninivorans* was chosen for this study because of its physiological characteristics, including its capacity to thrive at high temperatures (up to 48 °C), tolerate a wide range of pH values (from 2 to 10), and tolerate salt (up to 20% NaCl). When employing trash with varied composition and properties as the substrate, all of these qualities are desired (Haslett *et al.*, 2011).It was also investigated if 2,3,5,6-tetramethyl-1,4-phenylenediamine (TMPD) might be used as a redox mediator.In *an A. adeninivorans*-based MFC, the use of TMPD as an anodic mediator and $KMnO_4$ as a cathodic reducing agent resulted in a considerable increase in maximum power density, or 1.03± 0.06 Wm2. The measured values were rather close to the maximum power output reported by Ganguli and Dunn for their yeast-based MFC (Fishilevich *et al.*, 2009).

Fungi Used as a Cathode Catalyst

Electricity output in MFCs is significantly influenced by the cathode side arrangement as well as the electron receiver at the terminal used in the cathode chamber. Oxygen is known to be the main electron acceptor because it is readily available, free of hazardous chemicals, and has better catalytic potential. Various fungal species are used as cathodic catalysts in fungi-based MFCs (Simões*et al.,* 2019). *Trametes versicolor* and *Ganoderma lucidum* are two well-known fungus species that are employed in MFCs and have a higher electrical production. The most popular cathode for MFCs seems to be platinum-coated carbon electrodes that employ scattered oxygen as an electron receiver.Due to Pt's alteration with carbon electrolytes, the pace of reaction is increased while the stimulation of oxygen reduction is decreased. A bacterial population inside the cathode compartment that generates enzymes that essentially catalyze redox processes is necessary for the increase of cathode efficiency (Carbajosa *et al.,* 2010). By reducing the activation energy and increasing the reaction rate, the introduction of an appropriate catalyst can increase the efficiency of oxygen reduction. Pt-coated carbon electrodes that employ dissolved oxygen as the electron acceptor are the most popular cathodes used in MFCs. The oxygen reduction activation energy was greatly reduced and the reaction rate was boosted in carbon electrodes modified with Pt. However, there are significant disadvantages to using a platinum catalyst, including high prices and toxicity. Chemical catalysts cannot compare to the benefits of enzymes, which include biocompatibility, greater specific selectivity, efficiency in transformation, and activity in benign circumstances (Clauwaert *et al.,* 2007).

Trametes versicolor:

It is well known that *Trametes versicolor* produces laccase and ligninolytic enzymes efficiently an MFC's effectiveness in using white-rot fungus. T. versicolor was employed with glucose as the only source of carbon for the generation of bioenergy in the cathode compartment of the two MFCs. One of the two MFCs was filled with laccase as an improved catholyte for the controlled experiment, while the other was filled with a carbon fiber cathode (Wu *et al.,* 2012).The irreversible inactivation of the enzyme made it possible for disturbances in the system to be eliminated thanks to laccase production. A medium containing glucose as a carbon source and the cathode chamber of an MFC were both loaded with C. versicolor inoculum. Since ABTS had previously been utilized as a successful mediator that enabled the transmission

of electrons between the electrode and laccase, it was employed as a redox mediator. As controls, two other MFCs were used, one with a catholyte enriched with readily accessible laccase, and the other with a carbon fiber cathode (Karnicka *et al.*, 2008).

Challenges and Perspectives for Fungi-Based MFCs:

The fundamental challenge facing fungi-based MFCs, like bacterial MFCs, is a low power generation that is insufficient to provide the energetic self-sufficiency of such systems.However, in-depth investigation is needed to design the specific changes in electrode material and to identify and enhance the electron transfer mechanism of various fungal strains that are useful in energy production. The historical evolution of microbial fuel cell technology using fungi as catalysts is outlined in this paper, along with the many operational aspects that may be used to optimize this technology to increase total power output. The paper also offers strategies for overcoming the constraints of this technology by creating fungal-mediated MFCs with a variety of powerful strains and utilizing a variety of nanomaterials for improved electricity production.Future research on fungi-based MFCs should concentrate on improving the systems' capacity to produce power. Investigations employing single-chamber reactors with various electrode designs and materials can be used to achieve this. Chemical catholytes and mediators, such as ferricyanide, must be eliminated for fungi-based MFCs to be used in practical applications. Finding novel fungus strains with high-efficiency electricity production is therefore essential.

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CRISPR: An Advance Technique of Gene Editing

Taniya Sengupta Rathore, Sahana Singh, Sheenat Chawla, Humaira Jasmin
Dept. of Biotechnology, School of Science, O P Jindal University, Raigarh, Chhattisgarh- 469109
Corresponding Author: Dr. Taniya Sengupta Rathore
Associate Professor, Dept. of Biotechnology
School of Science, O P Jindal University
Email id: *taniya.rathore@opju.ac.in*
Taniya.sengupta@gmail.com

Abstract

For many years, gene therapy has shown promise in treating a range of human illnesses and abnormalities. The field of molecular biology has been completely transformed by the discovery of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), the mechanism of the CRISPR-based prokaryotic adaptive immune system (CRISPR-associated system, Cas), and its repurposing into a powerful gene editing tool.Additionally, the CRISPR/Cas9 site-specific nuclease system is widely used in many biological research fields, including the development of model cell lines, the identification of disease targets, the discovery of disease mechanisms, the creation of transgenic animals and plants, and transcriptional modulation. In this overview, we outline the background and fundamental workings of the CRISPR/Cas9 system and its forerunners (ZFNs and TALENs), as well as the lessons we've learned from previous attempts at human gene therapy and the most recent changes made to CRISPR/Cas9 to expand its capabilities beyond gene editing.Before effective in vivo human gene therapy is possible, the authors present a number of parameters that affect CRISPR/Cas9 efficacy and must be addressed. The most challenging obstacle to future CRISPR/Cas9 in vivo usage, transport, is then the focus. We describe the various CRISPR/Cas9 cargos and delivery vehicles, including physical delivery techniques (such as microinjection and electroporation), viral delivery techniques (such as adeno-associated virus (AAV), full-sized adenovirus and lentivirus, and non-viral delivery techniques (such as liposomes, polyplexes, and gold particles), and discuss the relative merits of each.We also look at a

number of technologies that, though they have not yet been reported for CRISPR/Cas9 delivery, show promise in this area. The immense therapeutic potential of CRISPR/Cas9 will only grow as the technology and methods for delivering it advance.

Key words: CRISPR/Cas9, Site Specific nuclease, Therapeutic Potential

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1. Introduction

Gene editing is a powerful technique that allows for the precise modification of genes in an organism's genome. Manipulation such as addition, deletion, modification and activation of gene can be done to alter cell fate by gene editing. This technology has revolutionized the field of genetics and holds great promise in various fields, including medicine, agriculture, and basic biology. Recent advancements in CRISPR/Cas9 genome editing have further propelled the field of gene editing, ushering in a new era in biotechnology. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology is a revolutionary tool in the field of genetic editing and genome engineering. This technology, based on a CRISPR-associated protein system, has garnered significant attention and has propelled advancements in the field of nucleic acid detection.

It is a versatile and user-friendly approach for precise and efficient cell manipulation. The simplicity of CRISPR technology allows for the targeting and modification of specific DNA sequences, making it a preferred choice for researchers in various organisms. The advent of CRISPR technology has provided a breakthrough in genome modification, particularly in biosynthetic gene clusters of *actinomycetes*.

CRISPR/Cas9 system holds great promise as a molecular tool for engineering non-model native hosts to heterologous production hosts in order to biosynthesize desired natural products.

2. CRISPR-Cas History: From an Unusual Repeated Sequence to Genome Editing Technology

*2.1 Discovery of CRISPR (***1993 - 2005 — FRANCISCO MOJICA, UNIVERSITY OF ALICANTE, SPAIN)**

The first CRISPR locus was described by Francisco Mojica in 1993, and his findings were published. He worked on them throughout the 1990s, and in 2000 he noticed that what had been suspected to be various repeat sequences actually shared a common set of characteristics that are now known as hallmarks of CRISPR sequences (he coined the term CRISPR through correspondence with Ruud Jansen, who first used the term in print in 2002). Jansen had been working on these sequences since the early 1980s. He stated in 2005 that these sequences matched bits of bacteriophage genomes (Mojica et al., 2005, Gostimskaya I. 2022). He accurately hypothesized that CRISPR is an adaptive immune system as a result of this discovery.

*2.2 Discovery of Cas9 and PAM (***MAY, 2005 — ALEXANDER BOLOTIN, FRENCH NATIONAL INSTITUTE FOR AGRICULTURAL RESEARCH)**

Streptococcus thermophilus, a newly sequenced bacterium that had a unique CRISPR locus, was the subject of Bolotin's research (Bolotin et al., 2005, Gostimskaya I. 2022). The CRISPR array resembled previously described systems, but several of the known cas genes were missing. Instead, it contained unique cas genes, including one that encoded Cas9, a big protein that they projected to have nuclease activity. They also observed that the spacers all contain a common sequence at one end and exhibit homology to viral genes. The protospacer adjacent motif (PAM), a part of this sequence, is necessary for target recognition.

2.3 Cas9 cleaves target DNA(December, 2010 — Sylvain Moineau, University of Laval, Quebec City, Canada)

According to research by Moineau and colleagues (Garneau et al., 2010, Gostimskaya I. 2022), CRISPR-Cas9 breaks double-stranded DNA at certain locations three nucleotides upstream of the PAM. Additionally, they verified that the CRISPR-Cas9 system only requires Cas9 as the necessary protein for cleavage.

2.4 Discovery of tracrRNA for Cas9 system(March, 2011 — Emmanuelle Charpentier, Umea University, Sweden and University of Vienna, Austria)

The team of Emmanuelle Charpentier provided the solution to the mechanism of natural CRISPR-Cas9-guided interference. They carried out small RNA sequencing on the CRISPR-Cas9-equipped Streptococcus pyogenes. They found that there is a second short RNA, which they named trans-activating CRISPR RNA (tracrRNA), in addition to the crRNA (Deltcheva et al., 2011, Gostimskaya I. 2022). They demonstrated that tracrRNA and crRNA form a duplex, and that Cas9 is guided to its target by this duplex.

2.5 CRISPR-Cas9 harnessed for genome editing(January, 2013 — Feng Zhang, Broad Institute of MIT and Harvard, McGovern Institute for Brain Research at MIT, Massachusetts)

Zhang was the first to successfully adapt crispr-cas9 for genome editing in eukaryotic cells (Cong et al., 2013, Gostimskaya I. 2022). Zhang and his team demonstrated targeted genome cleavage in human and mouse cells using two distinct cas9 orthologs they generated from *S. thermophilus* and *S. pyogenes*. Additionally, they demonstrated that the system could be set up to target numerous genomic locations and could activate homology-directed repair.

2.6. *DNA manipulation with the CRISPR–Cas9 system (in 2012 by American scientist JenniferDoudna, French scientist Emmanuelle Charpentier)*

The discovery of CRISPR/Cas9, often known as the genetic scissor, by Jennifer Doudna and Emmanuelle Charpentier later earned them the 2020 Chemistry Nobel Prize.

3. Classification of CRISPR-Cas system

CRISPR-Cas systems are classified into three major groups (I, II, and III) and eleven subtypes (I-A to I-F, II-A to II-C, and III-A to III-B) based on CRISPR locus arrangement and cas gene content. CRISPR-Cas systems demonstrate exceptional mechanistic diversity, especially for crRNA synthesis and interference, despite having a shared purpose in delivering adaptive immunity.The repeat sequences of the pre-crRNA are cleaved by the Cas6 nuclease family to produce tiny mature crRNAs in both Type I and Type III systems. Contrarily, Type II CRISPR-Cas systems process pre-crRNA transcripts by partnering a short trans-activating crRNA (tracrRNA) with the pre-crRNA's repeat region, then an endogenous RNase III cleaves the repeat region(Jiang et al., 2015, Makarova et al., 2015).

3.1. CRISPR- Cas system I

In Type I systems, mature crRNA binds to complementary DNA target sequences and recruits a trans-activating nuclease-helicase (Cas3) for unwinding and DNA cleavage via the CRISPR-associated complex for antiviral defense (Cascade) (Makarova et al., 2015).

3.2. CRISPR-Cas system II

The most basic CRISPR-Cas system is type II, which uses a single multidomain protein called Cas9 and a co-processed dual-tracrRNA:crRNA which instructs Cas9 to cleave target DNA sequences matching the RNA's 20-nucleotide guide sequence when combined as a chimeric single-guide RNA by joining the 3' end of crRNA to the 5' end of tracrRNAI (Barrangou et al., 2014). Type II systems are excellent prospects for a new generation of potent tools for genomic engineering because of their small enzyme machinery and easily programmable site-specific DNA targeting (Jongbeom Park et al. 2022, Makarova et al., 2015).

3.3. CRISPR-Cas system III

Large multiprotein assemblies called the Csm (III-A) and Cmr (III-B) complexes are used by type III systems to bind DNA or RNA that is directed by cognate crRNAs (Makarova et al., 2015).

4. The Science behind CRISPR

The CRISPR/Cas9 utilizes the CRISPR-associated protein system, specifically the Cas9 protein, to target and modify specific DNA sequences with remarkable precision and efficiency.RNA-mediated adaptive immune systems that are encoded by prokaryotic CRISPR-Cas genomic loci share several functional characteristics with eukaryotic RNA interference (Barrangou et al., 2014, Amemura et al., 1985). When foreign DNA sequences longer than 30 base pairs are incorporated into the host genome, acquired and heritable protection against bacteriophage and plasmids can start to develop. Together with CRISPR-associated (Cas) proteins, CRISPR-derived transcripts assemble to target complementary nucleic acids for apoptosis. Since its first application in eukaryotes in 2013, the CRISPR/Cas9 system has gained widespread popularity and has been extensively used in genome engineering in a variety of eukaryotic species (Hille et al., 2016).

In order to attack a bacterium, bacteriophages (viruses that prey on bacteria) inject their DNA through the bacterial cell membrane. Once within the cell, this bacteriophage DNA can rewire the invaded bacterial cell and induce it to produce additional bacteriophages.Prokaryotes have evolved ingenious defenses against viral infection and block plasmid transfer. CRISPR is one of these techniques.

The three steps of CRISPR-Cas adaptive immunity are spacer acquisition or adaptation, CRISPR-CasexpressioncrRNA biogenesis or, and DNA interference.

4.1. Spacer Acquisition or adaptation

During the acquisition stage, a short protospacer sequence from previous mobile elements is inserted into the CRISPR array as a new spacer.These acquired spacer sequences function as a genetic record of previous infections. Cas1 and Cas2 proteins appear to be ubiquitously involved in the spacer acquisition process, as they are found in practically all CRISPR-Cas types. The type III-C, III-D, and IV CRISPR-Cas systems are exceptions because they lack homologous proteins. The targeting sequence that is integrated into the CRISPR locus is not chosen at random. It has been shown that in type I, II, and V CRISPR-Cas systems, a small sequence known as the protospacer adjacent motif (PAM) positioned exactly next to the protospacer is critical for acquisition and interference. Cas9's PAM-recognizing domain is in charge of protospacer selection in type II-A CRISPR-Cas systems (Mojica et al., 2009). The absence of PAM recognition sequences in the direct repetitions

of bacterial CRISPR loci removes the possibility of self-targeting and self-cleavage by CRISPR-Cas systems, whereas changes inside PAM sequences allow the phage to avoid CRISPR immunity(Hille et al., 2016).

4.2 CRISPR-Cas expression or crRNA biogenesis

The CRISPR array is transcribed into a lengthy precursor crRNA (pre-crRNA) to facilitate immunization, which is then processed into mature guide crRNAs that include the invaders' remembered sequences. Members of the Cas6 family carry out the processing phase in type I and type III systems, producing intermediate species of crRNAs that are flanked by a short 5′ tag (Zetsche et al.,2015).The type I-C systems, which do not code for Cas6 proteins, serve as an exception. Pre-crRNA is processed by the protein Cas5d in this instance, producing intermediate crRNAs with an 11 nt 5′ tag. The mature crRNA species, which typically exhibits a hairpin shape in most type I systems, is produced by further trimming of the 3′ end of the intermediate crRNA by an unknown nuclease(Mojica et al., 2009). This mature crRNA species is made of a full spacer section (5′ end) and a repeat-portion (3′ end).Differential crRNA maturation occurs in class 2 CRISPR-Cas systems. TracrRNA is necessary in type II systems for the processing of pre-crRNA(Carteet al. 2008). With each of the pre-crRNA's repeats, this RNA's anti-repeat sequence promotes the development of an RNA duplex, which Cas9 stabilizes. The host RNase III then recognizes the duplex and processes it, producing an intermediate form of crRNA that goes through additional maturation by a still-unknown mechanism to produce the mature short guide RNA. The Neisseria meningitidis type II-C CRISPR-Cas system has an RNase III-independent mechanism. Each repeat in this instance contained a promoter sequence, some of which might start transcription and produce intermediate crRNA species.Although the crRNA:tracrRNA duplex's 3′ processing by RNase III was found, it was not necessary for interference. It has been demonstrated that Cpf1 serves two purposes in the type V-A CRISPR-Cas system during CRISPR-Cas immunity. Following an additional maturation event of unknown type, Cpf1 processes premature crRNAs and uses the processed crRNAs it has produced to cleave target DNA(Jiang et al., 2015).

4.3 DNA interference or target interference

Mature crRNAs are employed as guides to precisely interfere with the invasive nucleic acids in the last stage of immunity. Target degradation is accomplished in class I systems via Cascade-like complexes (CRISPR-associated complex for antiviral defense), but target interference in class II systems just requires a single effector protein.Type I, II, and V systems

specifically identify the PAM sequence that is positioned either upstream (types I and V) or downstream (type II) of the protospacer to prevent self-targeting.In type III systems, the 5′ tag of the mature crRNA, which must not base pair with the target to facilitate degradation by the complex, is used to distinguish between self and non-self.In type I systems, Cascade restricts invading DNA in a crRNA-dependent method and then enlists the nuclease Cas3 for target breakdown. Cas3 causes a nick to form on the foreign DNA before degrading the target DNA(Westra et al., 2012). In type II CRISPR-Cas systems, the effector protein Cas9 is guided to introduce a double-strand break in the target DNA by the tracrRNA:crRNA duplex. Cas10-Csm (types III-A and III-D) and Cas10-Cmr (types III-B and III-C) complexes, which can target both DNA and RNA, are part of the interference machinery of type III systems (Hille et al., 2016).

5. Genome Engineering

With a Type II CRISPR system, genome editing with CRISPR-Cas9 is accomplished. This system includes a ribonucleoprotein (RNP), composed of Cas9, crRNA, and tracrRNA, as well as an optional DNA repair template, when it is used for genome editing.

To transfect the target cells, CRISPR-Cas9 frequently uses plasmids that contain the components of the RNP, or the RNP is constructed before being added to the cells via nucleofection(Westra et al., 2012, Gee et al., 2017, Jiang et al., 2015). As the sequence that Cas9 employs to recognize and directly bind to particular sequences inside the host cell's DNA, the crRNA is specifically created for each application. Only in areas where editing is wanted must the crRNA bind. Each application's repair template is specially created since it needs to partially complement the DNA sequences on either side of the cut and contain the necessary sequence for insertion into the host genome.A single-guide RNA (sgRNA) can be created by bundling many crRNAs with the tracrRNA. In order to transfect this sgRNA into cells, it can be combined with the gene that produces the Cas9 protein and formed into a plasmid. There are numerous internet tools that can be used to help create efficient sgRNA sequences.

Table 01: Major Components and their functions of CRISPR system II (Asmamaw et al., 2021)

Component	Functions
crRNA	Contains a region that binds to tracrRNA (often in the form of a hairpin loop), generating an active complex, and the guide RNA that locates the proper section of the host DNA.
tracrRNA	creates an active compound by binding to crRNA.
sgRNA	Single-guide RNAs are compound RNAs made up of a tracrRNA and at least one crRNA.
Cas9(most commonly)	An enzyme that can alter DNA in its active form. Because of the DNA site recognition function of each enzyme, many versions exist with distinct functions (e.g., single-strand nicking, double-strand breaking, DNA binding).
Repair template	A DNA molecule that is employed as a template in the host cell's DNA repair process, allowing the insertion of a specific DNA sequence into the host segment that has been damaged by Cas9.

Cas9 and single-guide RNAs have been used to introduce site-specific double-stranded (ds) DNA breaks in eukaryotic cell genomes, which can then be repaired using non-homologous end joining (NHEJ) or homology-directed repair (HDR), resulting in site-specific and permanent genome modifications (Groenen et al., 1993, Jinek et al., 2014). Cas9 can be designed to target and/or cleave practically any target DNA location adjacent to a PAM motif by modifying the DNA target-binding sequence within the guide RNA. This simplified two-component CRISPR-Cas9 system provides researchers with a straightforward and effective tool for genome editing and gene regulation in a variety of organisms (Mojica et al., 2009).

6. Structure of CRISPR-Cas9

High levels of accuracy and straightforward design are features of CRISPR-Cas9. It is dependent on the target sequence and the protospacer adjacent motif (PAM) sequence for its specificity. The target sequence for each CRISPR locus in the crRNA array is 20 bases long. Typical crRNA arrays have a variety of distinct target sequences. By using the sequence to form bonds with base pairs on the host DNA, Cas9 proteins choose the proper site on the

host's genome (Ishino,et al.,2018). The sequence can be altered and independently produced because it is not a component of the Cas9 protein. Cas9 recognizes the PAM sequence on the host genome. It is difficult to change Cas9 to identify a different PAM sequence. The SpCas9 PAM sequence, for example, is 5'-NGG-3' and occurs around every 8 to 12 base pairs in the human genome, so this is ultimately not too restrictive. It is also often a fairly brief and generic pattern that occurs repeatedly at numerous locations throughout the genome (Jinek et al., 2014, Ishino et al., 1987).

After being put together into a plasmid and transfected into cells, the Cas9 protein uses the crRNA to locate the proper sequence in the DNA of the host cell and, depending on the Cas9 variant, either forms a single-stranded break or a double-stranded break at the necessary site in the DNA(Wiedenheft et al. 2012, Westra et al., 2012).The intended outcome is for the new sequence to be incorporated into the genome by the cell's native HDR process, which will use the given repair template. This new sequence is now a part of the cell's genetic makeup and is passed on to the cell's daughter cells after being incorporated. A tiny chemical and siRNAs working together to temporarily inhibit NHEJ and TMEJ can boost HDR efficiency to up to 93% while also preventing off-target editing (Jinek et al., 2014, Ishino,et al.,2018).

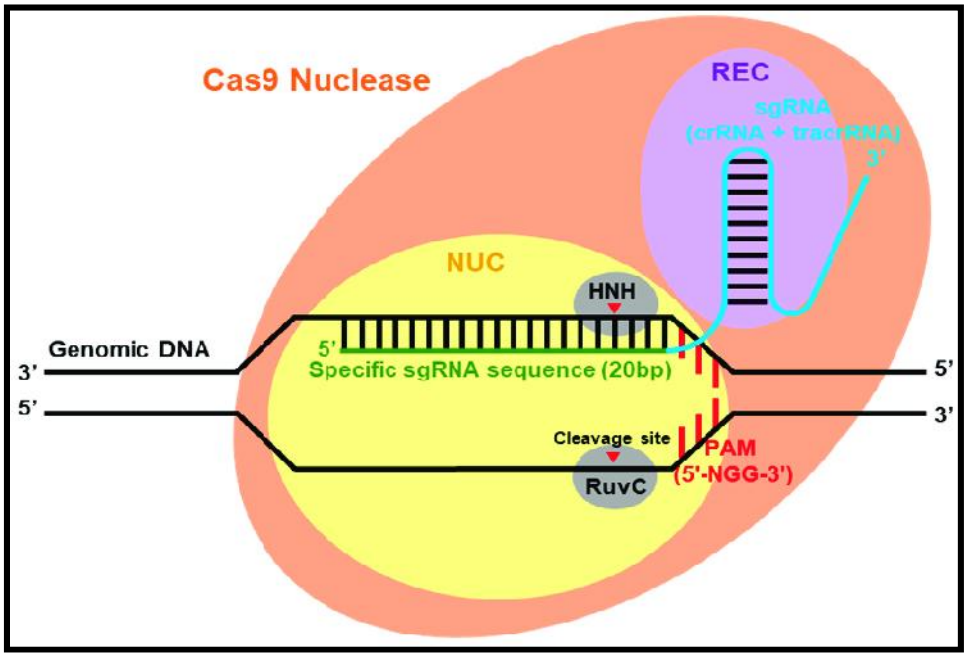

Fig 01: Structure of CRISPR-Cas9 (Jongbeom et al, 2022)

7. Applications of CRISPR

7.1. Transfection

Viral and non-viral techniques can be used to deliver Cas9, sgRNA, and related complexes into cells. It is a common approach to electroporate DNA, RNA, or ribonucleocomplexes, although it can have negative consequences on the target cells. The introduction of sgRNAs in association with Cas9 into cells has also been accomplished chemically, using transfection techniques based on lipids and peptides. Transfection has also been accomplished using delivery based on nanoparticles. Stem cells, neurons, and hematopoietic cells are examples of cell types that require more effective delivery methods, such as those based on lentivirus (LVs), adenovirus (AdV), and adeno-associated virus (AAV) (Guha et al., 2017).

7.2. Disease model

Cas9 genome modification has made it possible for the science of genetics to produce transgenic models quickly and effectively. In order to simulate the spread of illnesses and the cell's reaction to and defense against infection, Cas9 can be simply introduced into the target cells coupled with sgRNA using plasmid transfection.At any stage of an organism's development, CRISPR-Cas9 can be used to edit the DNA of living things in vivo and remove specific genes or even entire chromosomes. The human chromosomes 14 and 21, in embryonic stem cell lines and aneuploid mice, respectively, as well as the Y and X chromosomes of adult lab mice, have all been successfully removed in vivo utilizing CRISPR technology. This approach may be helpful in addressing genetic conditions like Down syndrome and intersex abnormalities that are brought on by aberrant chromosomal counts (Guha et al., 2017).

7.3 Biomedicine

CRISPR-Cas technology has been proposed as a therapy for a variety of human diseases, particularly those caused by genetic mutations.Its capacity to change specific DNA sequences makes it a tool with the potential to repair disease-causing mutations. Early animal research suggests that CRISPR-based therapies have the potential to treat a wide range of diseases, including cancer, progeria, beta-thalassemia, sickle cell disease, hemophilia, cystic fibrosis, Duchenne's muscular dystrophy, Huntington's disease, transthyretin amyloidosis[41], and heart disease.CRISPR has also been used to treat malaria in mosquitos, which has the potential to eradicate both the vector and the disease in humans (Guha et al., 2017).

7.4. Cancer

The creation of cell-based immunotherapies has made extensive use of CRISPR. In 2016, the first CRISPR clinical trial got under way. It involved extracting immune cells from lung cancer patients, using CRISPR to remove the PD-1 gene, and giving the modified cells back to the original patient.Using therapeutic gene editing for this purpose for the first time, doctors at Great Ormond Street Hospital in London were able to cure a 13-year-old British girl who had been diagnosed with an untreatable form of T-cell acute lymphoblastic leukemia in December 2022. She had undergone a six-month experimental treatment after other treatments had failed. The method involved first curing her of leukemia by reprogramming a healthy T-cell to kill the malignant T-cells, and then completely reconstructing her immune system from the ground up using healthy immune cells (Guha et al., 2017).

7.5. HIV/AIDS

HIV is retroactive, which means that it leaves an inactive copy of itself in the human genome. By creating guide RNA that targets the human HIV genome, CRISPR can be utilized to selectively delete the virus from the genome. This method has a drawback in that it calls for the elimination of the HIV genome from practically all cells, which can be challenging to realistically accomplish (Guha et al., 2017).

7.6. Blindness

CRISPR therapy for LCA10 (the most frequent type of Leber Congenital Amaurosis, the major cause of inherited childhood blindness) changes the patient's defective photoreceptor gene.The first patient volunteer in this US-based trial, sponsored by Editas Medicine, was given a low-dose of the medication in March 2020 to test its safety.

Enrollment for a high-dose adult and pediatric cohort of four patient volunteers each began in June 2021. In November 2022, Editas claimed that 20% of the patients treated experienced significant benefits, but that the resulting target population was too small to justify ongoing independent development(Guha et al., 2017).

7.7. Infection

"RNA-guided nucleases" based on CRISPR-Cas can be used to target virulence factors, genes encoding antibiotic resistance, and other medically relevant sequences of interest. As a result, this technology represents a unique

form of antibiotic therapy as well as an approach for manipulating bacterial populations (Guha et al., 2017).

7.8. RNA editing

Researchers stated in 2016 that CRISPR from a common oral bacterium may be used to modify RNA. Unlike permanent DNA editing, the effects of RNA editing, including potential off-target alterations in RNA, are temporary and not inherited. As a result, RNA editing is thought to be less dangerous. Furthermore, instead of introducing a foreign protein into the body, it may only require a guide RNA by exploiting the ADAR protein already found in the cells of humans and many other eukaryotes (Guha et al., 2017).

7.9. Others

Many facets of therapeutic strategies for lung cancer are investigated, such as target gene discovery, the development of animal tumor models, and the discovery of genes related to drug resistance. agricultural yield, quality, environmental stress, regulation of gene transcription, and the creation of transgene-free genome edited crops are all factors that are taken into account in the improvement of agricultural plants and study into the roles of genes (Guha et al., 2017).

8. Future aspects

Due to its ability to edit DNA to change target genes, activate tumor suppressor genes, silence oncogenes, and silence tumor resistance mechanisms for targeted therapy, CRISPR-dCas9-based artificial transcription factors (ATFs) may be employed in cancer therapy. Additionally, combining drug repurposing with CRISPR-dCas9-based ATFs could be an additional cancer treatment to lower cancer mortality. The purpose of this review is to discuss how repurposed medications may work better when paired with CRISPR-dCas9-based ATFs to treat cancer.

By carefully editing the targeted change of plant genomes, including model plants, food and horticulture crops, genome editing has altered the world of plants. Recent developments in the CRISPR/CRISPR/associated protein 9 (Cas9) genome editing tool have revealed it to be an effective targeted modification in the majority of fruit, vegetable, and ornamental plants, and because of its high accuracy and efficiency, it promises to speed up crop improvement.The effectiveness of CRISPR/Cas9 genome editing is influenced by a number of variables, including promoter sequence, RNA polymerase specificity, Cas9 expression, vector types, guide RNA expression, etc.

For a big section of the world's population, rice is a crucial crop because it provides the majority of their food and the majority of their income from agriculture. Recently, genome editing has been the focus of study, and it is now being used to good effect to raise the standard of rice. Rice genes may now be targeted and modified more easily in research using CRISPR genome editing technologies, which will help breeders create better rice types. The goal of current study is to increase the effectiveness of CRISPR genome editing tools even more in order to successfully change endogenous rice genes. These research suggest that rice genome editing is an effective and doable endeavor.

9. Controversies and Limitations

With the emergence of CRISPR technology, targeted editing of a wide variety of genomes is no longer an abstract hypothetical, but occurs regularly. As application areas of CRISPR are exceeding beyond research and biomedical therapies, new and existing ethical concerns abound throughout the global community about the appropriate scope of the systems' use. The fundamental ethical issues including the following:

1) The extent to which CRISPR use should be permitted

2) Access to CRISPR applications

3) Whether a regulatory framework(s) for clinical research involving human subjects might accommodate all types of human genome editing, including editing of the germline; and

4) Whether international regulations governing inappropriate CRISPR utilization should be crafted and publicized.

The off-target effect is the primary ethical concern raised by the National Institutes of Health (NIH) regarding GGE. Insertional mutagenesis and gene mutation could perhaps be caused by off-target gene mutation. Germline therapy's effects could be lethal, according to bioethicists and scientists who argue that genome editing is a new and unreliable technology and that little is understood about the systems governing gene control and embryonic development(Wiedenheft et al. 2012). Although CRISPR/Cas has shown to be a useful tool for therapeutic somatic applications, it has not yet developed to the point where it may be used to change the human genome for clinical reproductive purposes. Therefore, it is impossible to ignore the apparent long-term impacts. Human embryos subjected to genome editing run a significant risk of developing pathological conditions and impairments that could last a lifetime (Uddin et al., 2020).

10. Alternatives to CRISPR Cas9 gene editing technology

10.1. CRISPR-Cpf1 System

CRISPR-Cpf1 is a viable alternative to CRISPR Cas9 since it is much easier to insert into cells and tissues due to its smaller size. This is due to the fact that it only requires one RNA, whereas CRISPR Cas9 requires two. It also allows researchers to integrate DNA more effectively since it slices the DNA with more precision (Guha et al., 2017, Gee et al., 2017).

10.2Retron Library Recombineering (RLR) Platform

Recombineering is one of the gene editing methods that is used to introduce mutations quickly and without damaging DNA. A Retron Library Recombineering (RLR) platform was developed by researchers at Harvard Medical School and the Wyss

Institute for Biologically Inspired Engineering manufacture millions of mutations while simultaneously labelling them to allow for simultaneous screening (Guha et al., 2017, Gee et al., 2017).

10.3 Cas-CLOVER Nucleases

A single guide RNA is used by the CRISPR/Cas9 system to make genomic incisions in undesirable places. As an alternative to CRISPR/Cas9, Hera BioLabs has developed the high-precision Cas-CLOVER gene, which has negligible off-target activity. It employs two guide RNAs to accomplish this selectivity (Guha et al., 2017, Gee et al., 2017).

10.4 Zinc Finger Nucleases (ZFNs)

These were the first endonucleases that could detect and cleave specific DNA sequences. They are composed of around 30 amino acid modules that engage with nucleotide triplets and can detect lengthy DNA sequences, resulting in on-target specificity. NovoHelix provides gene editing services in animal models using ZFNs (Guha et al., 2017, Gee et al., 2017).

10.5 Transcription activator-like effector nucleases (TALENs)

These are restriction enzymes that have been created to cleave specific DNA sequences by fusing a TAL effector with any chosen DNA sequence. These TALENs can easily cut DNA at precise sites when paired with an appropriate nuclease. CROs such as Biocytogen provide TALEN design and validation services such as mouse model construction and genotypic identification (Guha et al., 2017, Gee et al., 2017).

11. Conclusion

CRISPR, a key tool in genome engineering, revolutionizes genetic editing and nucleic acid detection, enhancing the efficiency of genetic engineering processes and overcoming natural interference mechanisms, as demonstrated by Emmanuelle Charpentier's team.The large-scale application of an engineered form of the Type II Cas9 targeting complex for site-specific genome modification in mammals, plants, fungi, and bacteria has sparked worldwide interest in CRISPR-Cas systems over the last two years. The structural discoveries provided here lay the groundwork for understanding how Cas9 detects and cleaves target DNA, as well as for future study into target selectivity and cleavage efficacy. Cas9 variations, both natural and artificial, are increasingly being exploited for genome editing.

CRISPR-Cas9 is a highly accurate and straightforward design that relies on the target sequence and the protospacer adjacent motif (PAM) sequence for specificity. The PAM sequence is recognized by the Cas9 protein. The new sequence is incorporated into the genome by the cell's native HDR process, making it part of the cell's genetic makeup.

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Immobilized Enzymes

Sarika Kulkarni
Assistant Professor,
E-mail: *sarikakulkarni2017@gmail.com*
and
Dr. G. V. Mali
Associate Professor,
E-mail: *gajamali@rediffmail.com*
UG & PG Dept. Of Microbiology,
Bharati Vidyapeeth (Deemed to be University)
Yashwantrao Mohite College, Erandwane, Pune -411038

Abstract

The enzymes are used from centuries in various fields, which are having tremendous scope in among every field. To use almost all enzymes in a proper manner the major technique invented is immobilization of enzymes. This chapter mainly concerns about the immobilization of enzymes. Enzymes can be immobilized by using various methods such as carrier binding, cross linking and entrapment. Further the carrier binding can be classified into various categories such as physical adsorption, ionic binding and covalent binding.Although these methods of immobilization can affect the enzyme properties such as pH, Vmax and Km, the immobilized enzymes have been used from an age in various industries as well as for the diagnosis of diseases. Therefore these enzymes are contributing in the development of science and technology.

Key words: Immobilization, Methods, properties, industrial application.

1. Introduction

Enzymes are the substances or chemicals which increase the rate of reaction without itself undergoing any chemical change. They are required in very small quantity. Enzymes can be a catalyst or a biocatalyst. Catalyst are the enzymes which are non-specific, that is same catalyst can act in different reactions and their inhibitors are also non-specific whereas the biocatalyst function only in living organisms and they are substrate specific, inhibitor specific and stereo specific. These enzymes are used for diverse catalytic

applications but now a days a more interesting term introduced is ‘Immobilized Enzymes’. The immobilized enzymes offer an attractive application for industrial purpose by making re-use of a particular enzyme.

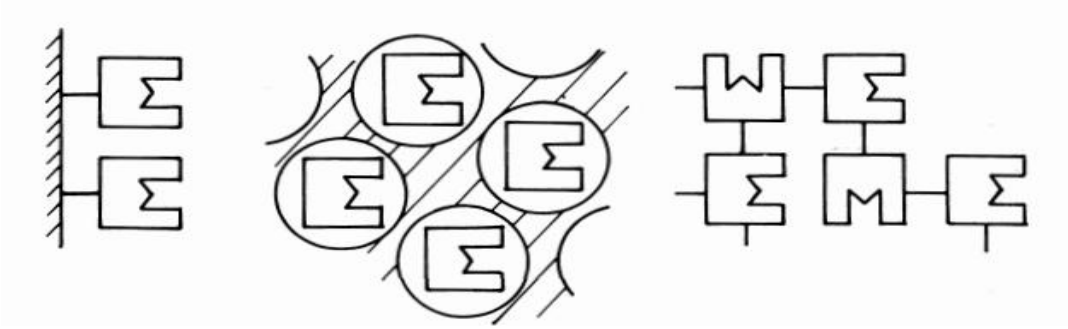
The term immobilization refers to restricting the movement of a substance partially or completely in a limited space. The immobilized enzymes can be defined as the enzymes which are either attached to any inert insoluble carrier or cross linked with each other without altering their chemical properties or without loss of enzymatic activities. Immobilization technique can be applied on living cells and enzymes. If the technique is applied on living cell, the bacterial cells can be lysed and then immobilization of extracted enzyme is carried out whereas when enzymes are available, they can directly be subjected to immobilization techniques.

1.1 Why Immobilized Enzymes?

The immobilized enzymes give diverse advantages such as it provides re-use of an industrially important enzyme for many reaction cycles. The loss of catalytic activity of an enzyme can also be prevented. It also provides stability to enzyme and protects the enzyme from degradation and deactivation.

2. Methods of Immobilization

The methods of immobilization are divided into three main categories such as carrier binding, cross linking and entrapment.



(a) **(b)** **(c)**

Fig 1. Supplementary representation for immobilization of enzyme (a) carrier binding (b) Entrapment (c) Cross Linkage

2.1 Carrier Binding

The carrier binding is a method in which enzyme is immobilized onto an insoluble solid support called as carrier. The enzyme binds to a carrier by one of the three methods such as physical binding mode, ionic binding mode and covalent binding mode.

2.1.1 Physical binding

Physical binding mode also called as physical adsorption. It is one of the simple methods of enzyme immobilization in which enzyme of an interest is physically adsorbed onto an inert insoluble carrier. In 1916, the first immobilized enzyme was produced by using this method by the Nelson and Griffin. They immobilized the enzyme invertase which breakdown the sucrose into glucose and fructose. The invertase was adsorbed on an inert insoluble carrier activated charcoal. This invention of immobilized enzymes was led to the use of some other inorganic carrier such as silica, clay and alumina. The physical adsorption creates weak interaction or non-covalent interaction between enzyme and its carrier. The non-covalent interactions involve Van der waals force and hydrogen bonds. As the interactions are non-covalent, the enzyme can be easily detached from the carrier. It can lead to changes in pH, ionic strength, substrate concentration and in the catalytic activity of an enzyme. The physical adsorption process is very non-specific which can accompany the binding of many other substance to the carrier.

2.1.2 Ionic Binding Mode

The modified method of physical adsorption is ionic binding mode as in both the methods non covalent interactions are used. The ionic binding mode gives more specificity in binding of an enzyme to an insoluble inert carrier which contains ion exchange resins. In this method, ion exchange resins are used as an inert insoluble carrier which may include CM cellulose and DEAE cellulose, in which CM cellulose acts as cation exchanger whereas DEAE cellulose acts as anion exchanger. The enzymes are attached to a matrix which is made up of carrier. The matrix may be positively charged or negatively charged which attaches to the negatively charged or positively charge enzyme respectively. The matrix is usually made up of any synthetic polymer or polysaccharide in which ion exchange centres are present.

The polysaccharide used in matrix are divided into two types such as natural and synthetic type. The natural polysaccharides contain starch and cellulose whereas the synthetic polysaccharide may contain poly vinyl alcohol, poly acryl alcohol and polystyrene.

Till the date various enzymes have been immobilized by using these methods. They are listed as in table 1.

Enzyme	Inert carrier
Hexokinase	Activated charcoal
Invertase	Activated charcoal
Amylase	Activated clay
Lipase	Alumina
Trypsin	CMC cellulose
β galactosidase	DEAE cellulose
Asparaginase	CMC cellulose
Catalase	Silica gel
Chymotrypsin	Hydroxy appetite
Acid phosphatase	Silica gel

Table 1. various inert polymers and inorganic carrier used as a solid support to enzymes for immobilization.

Advantages

a. It is very simple and cheap method.
b. Due to binding of enzyme to surface it may result in very little or no confirmational change in an enzyme.
c. No destruction of an enzyme.

Disadvantages

a. Physical adsorption interactions are non-specific which may be the cause of non-specific binding of another molecules.
b. The enzyme may escape from the carrier due to non-covalent interaction between enzyme and carrier.

Uses of Physical Binding Mode

a. Majorly used for vinegar production by immobilizing the *Acetobacter species* on an inert carrier wooden chip.
b. Used to produce the beer by using *Saccharomyces cerevisiae*, immobilized yeast adsorbed on polystyrene carrier which is covered with DEAE cellulose.

2.1.3 Covalent Binding

It is also called as covalent coupling. In this method enzymes are bound to an insoluble inert polymer with the help of functional group which are not involved in catalytic activity of an enzyme. The interaction between enzyme and the carrier are covalent which gives more stable and permanent linkage. The conditions should be mild for the formation of covalent bond to protect the enzyme and its substrate binding site from any substrate analogue. Therefore, the covalent binding or coupling occurs in the presence of competitive inhibitor in order to protect an active site of an enzyme. There are various types of functional group commonly used for the purpose of attachment in the active site of enzyme. They are amino group, sulfhydryl, phenolic, indole, thiol, imidazole, carboxyl and hydroxyl group.

Following are some of the reactive functional groups which are present in the enzyme for the purpose of attachment to the carrier.

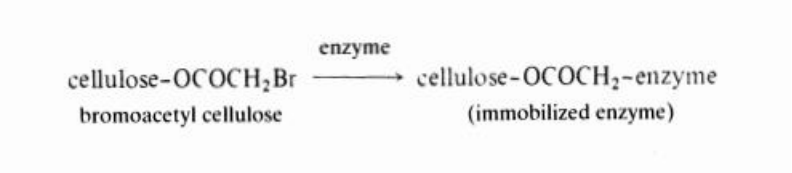
Phenol group of tyrosine, carboxyl group of acidic amino acid such as aspartic acid and glutamic acid, ε amino group of lysine and guanidino group of arginine and sulfhydryl group from sulphur containing amino acids such as methionine and cysteine.

As discussed previously the carrier are inert. Therefore, to carry out the reaction first step is to activate the carrier and then proceed for immobilization. There are various reactions to activate this inert carrier:

1. Acylation/ Alkylation
2. Reaction with aromatic diazonium salts with tyrosine and histidine
3. Reactions with heavy metals
4. Amide bond formation
5. Sulphhydryl exchange

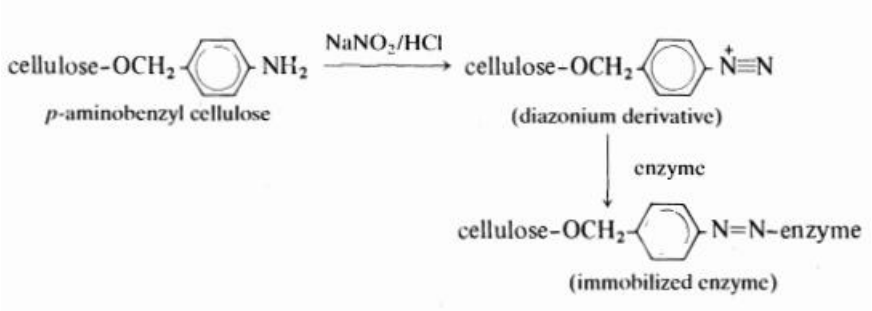
1. Acylation/ Alkylation

The acylation/ alkylation method is used for activation of polymer by using isocyanates, iso thiocyanate, halides, anhydrides and acyl oxide etc. which leads to immobilization of an enzyme. The examples in this category are enzymes such as ribonuclease, trypsin and chymotrypsin immobilized by using cellulose bromo acetyl bromide.



2. Reaction with aromatic diazonium salts with tyrosine and histidine

It is also called as diazonium coupling. The reaction is conducted when any diazonium salt reacts with functional group of proteins such as guanidino group, amino group, imidazole and phenol group. Room temperature is the favourable condition to carry out this reaction.

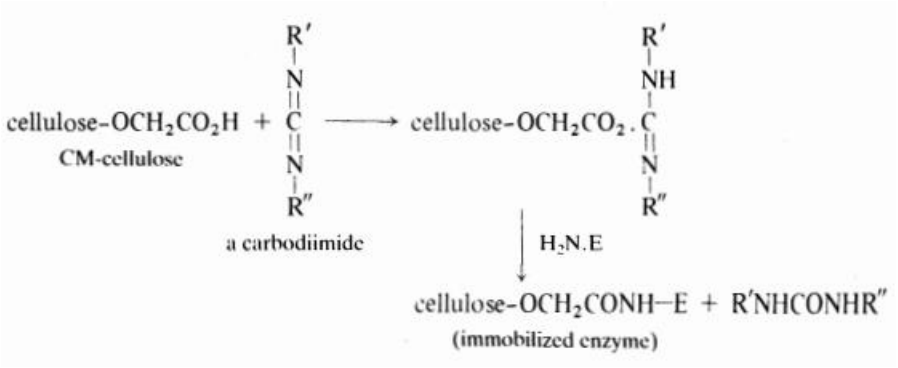


3. Reactions with heavy metals

The reactions are also termed as metal linked enzyme reactions. The heavy metals are toxic in nature but the transition from of metals can be used to activate inert polymers such as nylon, cellulose, borosilicate etc for example: $FeCl_3$, $TiCl_4$.

4. Amide bond formation

The amide bond formation occurs between the organic acid such as formic acid, malic acid carboxylic acid and the amine by using the reagent carbodiimide. This method usually used for the structures having carboxylic acid side chains.



5. Sulphhydryl exchange

As the name itself suggest sulphhydryl, the enzymes which contain sulphur containing amino acid such as cysteine, methionine are mostly used at acidic pH to immobilize the enzyme.

Advantages:

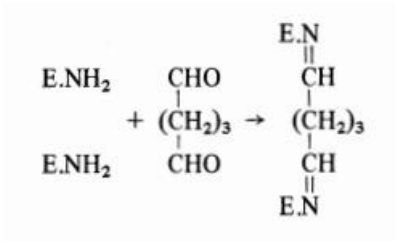
a. Enzyme do not leak upon binding to the support.
b. Provides stability to enzyme.

Disadvantages:

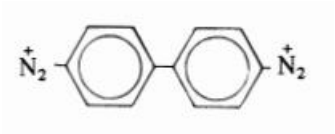
a. Enzymatic activity may lose.
b. The enzyme and matrix cannot be reused again.

2.2 Cross Linkage

The cross linkage is carried out by using cross linking agents such as glutaraldehyde in which two aldehyde groups will form bonding with free amino acid .This leads to the formation of Schiff's base.



Other cross-linking agents are phenol 2,4 Di sulphonyl chloride and 1,5 Di fluoro2,4 Di Nitro Benzene. One of the cross-linking agent is bis-diazo benzidine which contain two functional reagents which are responsible for immobilization activity.



Derivative of bis-diazo benzidine

In this cross linking is carried out between two enzyme molecules to immobilize it. The first immobilized enzyme carboxypeptidase A was prepared by Quiocho and Richards using the cross linkage. When the enzyme is present in high concentration, the intermolecular cross linking occurs. This results in an immobilization of enzyme as enzyme stabilizes in insoluble form. However,

when the enzyme is present in low concentration, intra-molecular cross linking occurs which makes the enzyme to remain in soluble state.

Advantages:

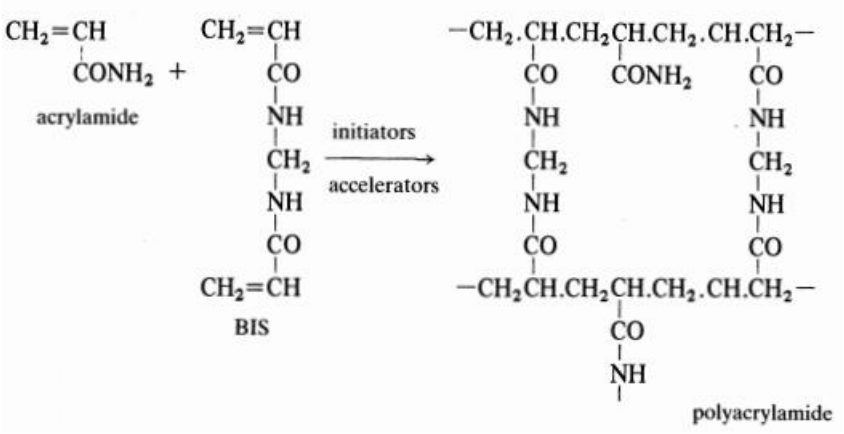
a. It provides maximum stabilization of biocatalyst.

Disadvantages:

a. Loss of enzymatic activity.
b. Not useful for packed beds.

2.3 Entrapment

It is also known as occlusion. The enzyme are entrapped or immobilized within grid or spaces in the gel. One of common example of gel is polyacrylamide. The gel can either be prepared from aqueous solution of acrylaminde or N,N methylene- bisacrylamide (BIS). It can also be used with the help of accelerators and initiators as shown in diagram:



The varying concentration of either acrylaminde or N,N methylene-bisacrylamide (BIS) can produce gel which is having difference in the pore size and strength that would directly affect the degree of cross-linking. If any enzyme is present in the solution of matrix, it would be directly entrapped within the lattice of gel or free spaces within the gel.

Another common method to immobilize enzyme by using the mechanism of entrapment is interfacial polymerization. In this, semi-permeable membrane of microcapsule is used to entrap the enzyme. The diameter of microcapsule is approximately 10-100 µm in diameter. The method was invented by the Chang and his team in 1964. In the emulsion technique , the

hydrophilic monomer and an aqueous solution of an enzyme is mixed with water insoluble organic solvent to form the emulsion.

Alternatively, hydrophobic monomer andan aqueous solution of an enzyme is again mixed with the same organic solvent used for the formation of emulsion. For example: the formation of nylon microcapsules takes place by either using hydrophilic monomer such as 1,6 hexamethylene diamine or hydrophobic monomer sebacoyl chloride. For both the hydrophilic and hydrophobic monomer the same organic solvent , cyclohexane-chloroform mixture is used. Moreover, another technique used for immobilization using the same procedure is the formation of liposome. The use of amphipathic lipids like cholesterol and phosphatidyl choline are employed which diffuse into the chloroform solution to which enzyme solution is added. The solution gets rapidly spread and leads to the formation of liposome. Liposome is the molecule, having the outer layer of lipids in which water droplets are enclosed.The enzyme can also be entrapped by using sodium alginate beads. When enzyme and sodium alginate mixed with calcium chloride, beads of calcium alginate are formed. The enzyme gets immobilized within the calcium beads.

Advantage:

a. It has wide applications in biotechnology and industrial purpose.

Disadvantages:

a. Enzyme may leak from the spaces.
b. Cannot be used for mass transfer.

3. Properties of Immobilized Enzymes

The properties of enzymes are depend on the binding between support and the enzyme. The interaction between enzyme and support affects the nature and characteristics of immobilized enzyme. The enzyme properties may vary in its original state and in its immobilized state which depends on the kind of method used. Generally specific gravity varies with free enzyme and immobilized enzymes. The immobilized enzymes usually have low specific gravity, due to the denaturation of enzyme. Moreover, the carrier has ability to form a new kind of microenvironment which could give the new characteristics to immobilized enzyme.

Immobilized enzymes also differ in their stability. After immobilization, stability of the enzyme can be either increased, decreased or may remain the same. It depends on how the immobilized enzyme responds to the new micro environment. The heat and storage of enzyme are also responsible for altering the stability of enzyme.

Once the enzyme gets immobilized, the optimum pH of the enzyme can be changed as far as by two units in response to the new micro environment. In the duration of 1964-1970, Goldstein and colleagues invented that if the carrier is anionic, the optimum pH of enzyme shift to alkaline whereas if the carrier is cationic , optimum pH shifts to acidic. It depends on the electrostatic field provided by the carrier for immobilization.

Apparent Km can also be affected by the electrostatic force of the carrier or the charge present on the carrier. When the carrier used is of opposite charge as that of the substrate the activity gets decreased. When the substrate is positively charged and carrier is negatively charged the activity of enzyme decreases significantly. For example-In one of the positively charged substrate Benzoyl L Arginine Ethyl Ester immobilized with polyanionic carrier CM-cellulose, apparent Km decreases 10 times due to the presence of carboxyl group. Moreover, when carrier and substrate used with same charge the apparent Km increases. For example- the substrate creatine kinase and its carrier CM-cellulose both have same charge which increases the apparent Km 10 times of the free enzyme.

4. Applications of Immobilized Enzyme

The enzyme immobilization is world wide attraction now a days in order to re-use the enzymes, to carry out complicated reactions. The immobilized enzymes are used in almost every field to enhance the product recovery.

Industrial production: The immobilized enzymes are used for industrial production of antibiotics, beverages, amino acid etc. The first application of immobilized enzyme for industrial production was the production of L aspartic acid. After the first experiment on L aspartic acid many products such as high fructose syrup, L malic acid were produced on industrial scale. Moreover, the immobilized enzymes are used for the conversion of penicillin V to 6 amino penicialic acid with the help of *Fusarium Sp.*

On industrial scale, the enzyme penicillin acylase is mostly used for the production of semisynthetic penicillin. Invertase is also used for the hydrolysis of sucrose into glucose and fructose. In the leather industry also immobilized enzymes such as various proteolytic enzymes are used to make the skin soft.

Researchers: Besides the natural polymer such as cellulose, gelatine, alginate and agar, some of the synthetic polymer such as polyacrylamide is used as a gel by the researchers. Now a days researchers have more belief in the immobilized enzyme to increase the productivity of the various enzymes such as HRP (Horse Radish Peroxidase) which is used in blotting and various proteases which are responsible for cell lysis.

Biomedical application: Immobilized enzymes are also used in medical field to diagnose and to treat the various diseases. They are used to treat the Leukaemia where immobilized enzymes directly act on the oncogenic site. For this, an exogeneous asparaginase is requiredwhich is isolated from bacteria. The leukemic cells grow on the exogeneous asparagine which can be lysed by the enzyme.

Another important enzyme hyaluronidases is used to treat various dental caries whereas the glucose oxidase is used to detect the presence of glucose in urine to diagnose the diabetes. Some of the patients have penicillin hypersensitivity which can be treated by using the penicillinase. Moreover, the lysozyme enzyme which is present in saliva, tears and body fluids also is used as protective barrier against bacteria.

Food industry: The immobilized enzymes such as cellulase, pectinases are used for the production of various food products such as jam, jellies and production of syrup from various fruits and vegetables.

Textile industry: They are also used in scouring to remove the dust and dirt from cloths, bio-polishing of cloths and for sizing of cloths.

Detergent industry: Lipase immobilized enzyme is used to remove the dirt from cloths.

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Soil Metagenomics - Concepts and Applications: A Review

Priya Sundararajan[1], Shanmuga Priya Ramasamy[2]*
[1]Research Scholar, Department of Microbiology,
PSG College of Arts & Science, Coimbatore, Tamil Nadu, India,
E-mail: *priyasundararajan2509@gmail.com*
[2]*Associate Professor, Department of Microbiology,
PSG College of Arts & Science, Coimbatore, Tamil Nadu, India,
E-mail: *priyajasper@gmail.com*
*Corresponding author: Dr. Shanmuga Priya Ramasamy,
Associate Professor, Department of Microbiology,
PSG College of Arts & Science, Coimbatore, Tamil Nadu, India;
E-mail: *priyajasper@gmail.com*

Abstract

Soil acts as a reservoir of both known and unknown microorganisms. The abundance of microbes residing in the soil ecosystem is more diverse than any other ecosystem. The soil ecosystem is also found to be a prominent source for screening various ideal secondary metabolites. Despite global importance and extraordinary diversity, the soil ecosystem has been notoriously challenging to study. Most microorganisms are not cultivable with standard procedures. At present, the metagenomic study is known as a pioneering technology that successfully explores a complete genomic sequence of microorganisms whereby the structural and functional properties of microbes can be determined promptly. In this perspective review, we discussed how metagenomic study pioneers a way for studying soil ecosystems. It also critically reflects the potential applications pertaining to studying soil metagenomics.

Keywords: Soil ecosystem, Metagenomics, Terragenome, Metaphenome, Applications

1. Introduction

The soil is considered to be a biologically complex ecosystem that contains more than millions of microorganisms. These microorganisms are found to act as a key component in a tropical ecosystem. Microbes play a significant role in maintaining soil health, plant productivity, disease management, etc., and they also play a crucial role in climatic change (Dubey *et al*., 2019). Microbial diversity has been traditionally determined with the help of laboratory cultivation techniques. However, only the least quantity of microbial diversity is explored successfully. The culture-dependent and culture-independent methods used to further exploitation of higher taxonomic ranks reside within microbial diversity. Even though it is stated that microbes are responsible for climate change, soil and plant health, and biogeochemical cycling, these cannot be identified using in-vitro techniques. Due to the limitations persisting within cultivation techniques, the metagenomic approach is widely recommended for studying microbial diversity. Metagenomics is one of the modern genomic methods used for the direct study of microbial communities present in the environment sample (Ghazanfar *et al*., 2010; Alteio *et al*., 2020). Initially, soil metagenomics was used to construct libraries, which are then further subjected to sequencing to produce a desired product. Later, it is potentially used for the identification of functional properties of the complex microbial population. The metagenomic analysis has been done via a series of steps, including DNA extraction, Library construction through Cloning into a vector and host cell transformation, and screening of transformants for structural and functional properties. The collection of sequences from metagenomic data and databases that offer consistent storage for metagenomes is challenging as it reflects the quality and quantity of data (Myrold *et al*., 2014; Felczykowska *et al*., 2015).

2. Microbial habitat in Soil

Soils are the primary form of all terrestrial ecosystems and act as a residence of the vast biotic diversity of microbes, insects, annelids, plants, etc. Among various biotic forms, microorganisms play a crucial role in maintaining the soil ecosystem (Aislabie *et al*., 2013). The soil environment is complex and harbors different types of microbes. Basically, microorganisms present in soil are divided into two groups. Group I includes prokaryotes like Bacteria, whereas Group II includes eukaryotes like fungi, viruses, and protozoa. The growth of microbes is greatly influenced by various environmental factors like pH, temperature, oxygen, moisture content, and availability of nutrients (Hattori, 1977; Tecon & Or, 2017). Microbes perform certain biological

activities in soil like decomposition, degradation, detoxification, nitrogen fixation, and transformation of macro and micronutrients. It helps in retaining soil structure and fertility, contributing to the stability of soil, and thus preventing soil erosion. Microorganisms, mainly bacteria, benefit plants by transferring nutrients from soil, which is away from the reach of plant roots. The disease-causing microbes have been attracted recently as they play an antagonistic property by inhibiting harmful microbes present in the soil. Thus, the health of plants is maintained promptly with the help of soil microorganisms (Handelsman *et al*., 1998; Clegg & Murray, 2002; Bhattarai *et al*., 2015).

3. Detection of soil microbial diversity

The microbial diversity of soil was detected through culture-dependent and culture-independent techniques. Culture-dependent methods are conventional techniques used for more than a hundred years to measure soil microbial composition. Culture-independent methods, including molecular techniques, have been developed to observe the existence of a large phylogenetic group of microbial strains present in soil (Zhang & Xu, 2008).

3.1 Culture-dependent methods

3.1.1 Serial dilution, plating, and culturing methods

It is the most traditional method for assessing soil microbial diversity. It relies on selective or differential media by which microbial species are retrieved, and subsequent viable counts or colony-forming units (CFU) are determined. It also provides information about the cultivable microbial population present in the heterotrophic segment of soil (Fakruddin *et al*., 2013; Bhatia *et al*., 2015).

3.1.2 Community-level physiological profile

This technique is used to analyze soil microbial communities based on their carbon source utilization patterns. It is facilitated through a taxonomic system, BIOLOG® by which carbon substrate utilization is detected through the reduction of tetrazolium dyes that results in characteristic colour change and are quantified spectrophotometrically (Hill *et al*., 2000).

3.2 Culture-independent methods

3.2.1 Fatty acid pattern profiling

It is said to be the quantitative method for accessing microbial communities in a specified environment at a particular time. Phospholipid fatty acids are present in membranes of all microorganisms, and each has a unique characteristic fatty acid pattern. In order to perform fatty acid profiling, fatty acids have to be extracted from soil microorganisms. The extracted fatty acids are subjected to exchange columns and are methylated and further detected with the help of Gas Chromatography. The fatty acid profiling can be done by the Fatty acid methyl ester (FAME) technique for detecting both polar and non-polar fatty acids, whereas Phospholipid fatty acids (PLFA) analysis is done for the detection of polar fatty acids (Marschner, 2007; Willers *et al*., 2015).

3.2.2 Density Gradient Gel Electrophoresis and Temperature Gradient Gel Electrophoresis

To determine the structure of the bacterial community, DGGE (Density Gradient Gel Electrophoresis) and TGGE (Temperature Gradient Gel Electrophoresis) could be performed. However, they could be used only when there is a higher concentration of microorganisms in the soil. This method is based on the electrophoretic separation of DNA upon the difference in melting behaviour in a gradient either through the usage of a denaturing agent or by increasing the temperature. It is also used to assess the active part of a community based on RNA-derived sequence analysis or functional gene sequence analysis that encodes proteins isolated from soil microorganisms (Valaskova & Baldrian, 2009; Rincon-Florez *et al*., 2013).

4. Steps involved in metagenomic analysis

4.1 Extraction of DNA

In order to isolate DNA from an environmental sample, either direct or indirect method was employed. For direct isolation, an in-situ lysis extraction technique has been used. It relies on the complete lysis within the soil matrix, thereby maximal yield of DNA could be achieved within a stipulated time. In the indirect isolation method, the soil matrix is initially dispersed to isolate bacterial cells. Followed by dispersion, cell lysis is performed through physical, chemical, or biological methods, showing the maximal yield of DNA. The extraction of DNA can be influenced by various factors, including types of environmental samples, DNA size and future use of the same. Hence, the selection of either the direct or indirect method of DNA extraction is based on the above-said factors (Felczykowska *et al*., 2015; Nesme *et al*., 2016).

4.2 Purification of DNA

The DNA is extracted from soil samples that are largely contaminated with humic and fulvic acids. To purify DNA from environmental contaminants, ion exchange chromatography or size exclusion chromatography is used. Gel electrophoresis can also be used to purify DNA from humic acid. However, the purification of DNA may lead to loss of DNA and minimize the process efficacy. Thus, a suitable method of purification and extraction should be opted. The pretreatment of soil by washing with chelating agents, salts or surfactants are also highly recommended for the extraction of DNA without any contamination and also prevents the loss of DNA (Kakirde *et al*., 2010; Lombard *et al*., 2011).

4.3 Construction of Metagenomic libraries

Followed by the extraction and purification of DNA, a metagenomic library is constructed for the recovery of biologically important metabolites. The steps involved in the construction of the metagenomic library, includes DNA fragmentation, vector insertion and transformation into a host. The small insert libraries with plasmid vector is a classical approach for library construction. But in this process, large gene clusters or operons are not detected. In order to overcome this limitation, large insert libraries are used with the help of cosmid or fosmid or BAC (Bacterial Artificial Chromosome) vectors. The vectors are selected based on the size and quality of the DNA to be isolated from the soil sample (Kimura, 2006; Simon & Daniel, 2010).

5. Screening of Soil metagenomics

Structural or functional screening methods could be employed to screen the gene of unknown function from unculturable microbes (Uchiyama & Miyazaki, 2009).

5.1 Functional-based screening

A large number of novel enzymes with unique metabolic activities were also identified with the help of various function-based metagenomic screening methods. In the agar plate technique, novel hydrolytic and non-hydrolytic enzymes have been discovered. The agar plate method is based on the production of fluorophore or chromophore upon incubation with the fluorophore or chromophore substances. This method is often used for screening the genes that are responsible for resistance to toxic substances like antibiotics, heavy metals, etc. In Fluorescence Activated Cell Sorting (FACS) screening, cell selection is performed based on cell shape, size, and fluorescence. In FACS screening, the metagenomic library is transformed into

the host cells, which harbours the reporter genes. Then, the gene products of the metagenomic library activate the expression of reporter genes with the help of transcriptional regulations or post-translational modifications. FACS is widely applied for high throughput screening of metagenomic clones, as it can be used to identify the biological activity within a single cell (Felczykowska *et al*., 2015; Kumar *et al*., 2015; Ngara & Zhang, 2018).

5.2 Sequence-based screening

Polymerase Chain Reaction (PCR) is the most used technique for sequence-based screening of soil genomes. The primers are designed to study the environmental genes from different metagenomic samples. Quantitative PCR enables us to study the metabolic activity of the desired gene through transcript analysis and provides information regarding the relevant key genes present within ecological samples. Microarray technology in metagenomic analysis was effectively implemented for functional gene identification. In this technique, the nucleic acids of the metagenome are isolated and labelled with a fluorescent dye, washed, and then the sequence is hybridized using a corresponding complementary probe and immobilized on a slide. The detection of a hybridized gene can be done with the help of a laser scanner, which detects the intensity of fluorescence signals on the hybridized spot (Sebet *et al*., 2003; Daniel, 2005).

Illumina sequencing is a sequencing-by-synthesis technology employed for the screening of metagenomes. The adapter sequences are ligated with DNA fragments to confirm the hybridization with oligonucleotides on the surface of the flow cell, and numerous clusters are formed due tothe amplification of fragments. By incorporating the differentially labelled reversible dye terminator bases, a fluorescent signal is produced and recorded by a camera. AB SOLiD system works based on sequencing-by-ligation, where the adapter sequences are added to fragmented DNA for immobilization and individual amplification on separate beads. The beads are then enriched with DNA amplicon, which is attached to the surface of a glass slide. Then, the sequencing primer is annealed, and a set of fluorescent oligonucleotides are hybridized with DNA. If the hybridization is matched, the corresponding fluorescent signal is recorded (Leis *et al*., 2013).

Next Generation Sequencing (NGS) is a high-throughput screening method in which the sequence of metagenomes can be analyzed and compared with other genomes available in the public database. In order to calculate inter-genomic distances of sequenced genomes, the genomes were cut into small fragments in in-silico. Then, the High-scoring Segment Pairs (HSPs) between

two genome sequences are determined using the BLAST algorithm. Alternative to BLAST, MUMmer an ultra-rapid alignment tool, can be used in which Maximally Unique Matches (MUMs) between the genome sequences can be identified. Metagenome analysis through NGS has potent lead to genetic diversity, population structure, and ecological function of uncultured microorganisms in the environments (Yi, & Chun, 2015).

6. TerraGenome

Numerous microorganisms were present in the soil. For instance, 1 gram of soil contains more than 10,000 bacterial species. Many of these cannot be isolated and cultured by standard techniques (Scholater *et al*., 2018). Advances in high-throughput gene sequencing technology in association with bioinformatics tools possibly help in studying soil microbial metagenomics of terrestrial ecosystem. TerraGenome is an international public consortium that aids in collaborating with various scientific communities to annotate and sequence soil microbial metagenomes around the world. It was recommended by the U.S National Research Council to share sequence information with respect to soil metagenomics. TerraGenome encourages various organizations to uncover the taxonomic, structural, and functional diversity of soil microbiota (Vogel *et al*., 2009; Pascal Simonet, 2009).

7. Soil metaphenome

Metaphenome is defined as the expression of functions encoded in metagenome or microbial genome and environment. In other words, a study on the relationship between metaproteomics with metabolomics provides an overview of the metaphenome of soil. The metaphenomic study confronts researchers due to its high complexity with temporal and spatial variability (Mayur Naitam & Abiraami, 2019; Madawala, 2019; Thiele-Bruhn *et al*., 2020). The knowledge of metaphenomes is considerably significant for understanding the functions of microbial communities in various ecosystems, including soil. The soil metaphenome pertains towards a highly structured soil environment. In order to predict the physiology and metabolic interactions of community members residing in soil, the distribution of microbes and resources should be known. It is quite complex to understand the physiology of interacting organisms to various environmental factors prevailing in the soil ecosystem. The community metaphenomic response is the collection of physiological responses of microbes to the environment, which includes genetic regulation and cell-to-cell interaction. The properties of microbial synthesis to genes and gene products in soil metaphenome analysis remain challenging (Jansson & Hofmockel, 2018; Chowdhury *et al*., 2019).

8. Application of Soil metagenomics

Soil is one of the most diverse and challenging environments. It holds infinite sources that may lead to the discovery of ideal products like bioactive compounds, bioprocess metabolites, enzymes, etc. An approach towards soil metagenomics helps in revealing novel products which could be exploited for various industrial and agricultural applications.

8.1 Soil health and agriculture

Microorganisms present in the soil are considered to be an essential source in maintaining soil health. Soil metagenomics is found to be a promising approach for exploring microbial communities present in the soil. Soil microbiota plays undisputable roles in carbon sequestration, biogeochemical cycling, and plant growth (Dubey *et al*., 2019). Most of the microbes present in the rhizospheric region of soil are able to produce biosurfactants, which could be widely used in improving soil quality. The biological activity of soil could be determined upon the estimation of dehydrogenase enzymes like β-glucosidase, chitinase, arylsulphatases, phosphatases, etc., produced by microorganisms. β-glucosidase is the indicator of soil quality which has the capacity to stabilize organic matter present in the soil. It also decomposes β-glucoside present in plant debris. Chitinase is an antimicrobial enzyme secreted by microbial strains, that are involved in controlling soil-borne pathogens. In the external environment, bacteria secrete the enzyme arylsulphatases, which is responsible for hydrolyzing sulphate esters present in the soil. Phosphatase plays a crucial role in the biogeochemical cycling of phosphorus and is said to be a good indicator of soil fertility. The quantity of these enzymes' activity represents the biological capacity of soil for the enzymatic conversion of the substrate, and these enzymes play an essential role in maintaining the ecology of microorganisms in the soil. The biogeochemical cycle of carbon, nitrogen, and sulphur also helps in maintaining soil fertility. Further, toxic compounds present in soil are microbially degraded by utilizing it as a nutrient source for microbial growth (Myrold *et al*., 2014; Sabale *et al*., 2019). In addition to this, sustainable agricultural production, soil microorganisms play a significant role in promoting plant growth, protection against stress, and defense responses in plants. A study on the relationship between plant and soil microbe's aids in the proper designing of crop systems. Moreover, the genes encoding for vitamin biosynthesis that enhance plant growth (eg. Biotin) have also been identified with metagenomic analysis. Thus, soil metagenomics helps in the formulation

of biofertilizers and biopesticides (Ghazanfar *et al*., 2010; Goel *et al*., 2017; Nelkner *et al*., 2019).

8.2 Exploring Antibiotics

In recent years, antibiotic resistance to existing antibiotics has become a serious threat. It urges researchers to identify novel antibiotics with potent antimicrobial activity. Metagenomics have a sustainable impact on isolating and identifying the unusual antibiotics from microorganism present in soil samples (de Castro *et al*., 2014). Certain enzymes like amidases are used in the biosynthesis of β-lactam antibiotics. Turbomycin A and B is an antibiotic discovered from the metagenomic library of soil microbial DNA. The anticancer compound indolotryptoline encoded by gene bor from soil exhibited good activity against tumor cell lines (Yashir Bashir *et al*., 2014). While screening soil metagenomic studies, it was found that the production and synthesis of N-acyltyrosine antibiotics by soil microflora leads to the development of novel antibiotics. Thus, soil metagenomics not only identifies antimicrobial compounds but also determines antibiotic resistance gene present in soil microbiota (Ghazanfar *et al*., 2010).

8.3 Bioremediation

Due to rapid urbanization and industrialization, harmful wastes have been dumped in soil resulting in soil erosion. Bioremediation through metagenomic strategy is an eco-genomic tool to detoxify contaminants present in terrestrial ecosystems. Understanding the difference between contaminated sites and non-contaminated sites is required and can be explored through soil metagenomics. Either microorganisms alone or else through their symbiotic relationship with plants can degrade a wide variety of hydrocarbons, aromatic compounds and heavy metals present in the environment (Devarapalli & Kumavath, 2015; Malla *et al*., 2018). Metagenomic research in bioremediation aids promptly in the characterization of microorganisms involved in bioremediation. The biodegradation pathway and novel gene families responsible towards bioremediation could be determined through metagenomics. Many researchers have reported on the construction and screening of metagenomic libraries to find genes involved in bioremediation through mRNA transcriptional profiles (Bharagava *et al*., 2019). This enables us to understand the process involved in bioremediation through the screening of microorganisms involved in bioremediation process from the metagenomic library (Techtmann & Hazen, 2016).

9. Conclusion

Soil microbial communities are essential factors of agroecological ecosystems that influence soil fertility, nutrient yield, and plant productivity. Metagenomics has a profound effect on studying microbial diversity in the soil ecosystem. It contributes towards various fields, including agriculture, pharmacy, ecology, etc. This review has addressed potential applications of metagenomics in the tropical soil ecosystem. The metagenomic approach provides researchers with a deeper insight into microbial diversity and functions present within the heterogeneous ecosystem. A study on soil physicochemical properties is crucial for further understanding the changes associated with the soil microbial community, their mechanisms, and their abundance. Nevertheless, this technique requires high-cost technologies and interdisciplinary experts. Further addressing these technological challenges pertaining towards metagenomics will promote a better understanding of soil ecosystems and is highly recommended in the near future.

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Panchagavya – A key tool in agriculture

Muniasamy[1], S. Padmavathi[1] M, Rooba Lakshmi[1] S, and P. Ponmanickam[2]

[1]Department of Microbiology, [2] Department of Zoology
Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi – 626 124.
E-mail: *ayya.mari@gmail.com*

Abstract

Panchagavya, a liquid organic fertilizer derived from cow by-products, has gained significant attention in agriculture due to its positive impact on soil health, plant growth, and crop yield. This natural formulation contains a diverse blend of beneficial microorganisms such as lactic acid bacteria, yeast, actinomycetes, photosynthetic bacteria and certain fungi. The application of panchagavya enhances soil fertility, improves nutrient availability, and promotes the growth of beneficial soil microbes. It also stimulates plant growth, leading to better crop morphology, increased yields, and improved resistance to diseases and pests. Panchagavya serves as an effective tool in organic farming, providing a cost-efficient and eco-friendly alternative to synthetic or chemical fertilizers. Its use has shown promising results in promoting sustainable agriculture and producing high-quality, chemical-free food products. Emphasizing the significance of natural practices, panchagavya holds the potential to contribute significantly to organic cultivation and overall agricultural development.

Key Words: Cow, fertilizer, organic farming.

1.0 Introduction

India is an agricultural country, where the modern agriculturalists utilized the chemical fertilizers for modern agriculture and nowadays, its adverse impact of on soil and plant health has led to a surge in demand for organic farming in recent years. This is driven by the recognition of natural based fertilizers for its inherent advantages, as it sustains crop production while also maintaining dynamic soil and nutrient status, promoting a safe environment. Currently, organic agriculture is recognized as a production management system that encourages biological activity. In India, the practice of organic farming has historical roots, dating back to ancient texts like the 'Vedas,' which mention the use of 'panchagavya' in agriculture. Panchagavya is

a concoction made from a blend of five products derived from cows that is, milk, curd, ghee, urine and dung. The Vedas and Vrikshayurveda extensively listed the applications and elaborated uses of panchagavya (Natarajan, 2002).

Foliar fertilization is a simple and effective method of providing nutrients to crops (Alexander and Schoeder, 1987). Panchagavya is popular most in organic farming in India and most of the states.In recent years, organic farming has gained increasing attention and yielded better results, mainly due to the negative impacts of chemical inputs. According to the United Nations Food and Agricultural Organization (FAO), organic agriculture expanded to cover 37.2 million hectares in 2011, which is three times more than the area in 1999. Organic farming strictly prohibits the use of synthetic pesticides, inorganic fertilizers, genetically modified organisms, and other chemicals. Instead, it relies on bio-fertilizers and conventional organic formulations derived from organic materials or waste to nourish the plants and maintain soil health (Badgley *et al.*, 2007).

In Panchagavya, effective microorganisms (EM) refer to a combination of naturally occurring and beneficial microorganisms that play a significant role in enhancing soil fertility. These microorganisms include lactic acid bacteria, such as *Lactobacillus*, yeast like *Saccharomyces*, actinomycetes such as *Streptomyces*, photosynthetic bacteria like *Rhodopsuedomonas*, and certain fungi like *Aspergillus*. This diverse blend of microorganisms works synergistically to promote soil health and contribute to the overall well-being of the agricultural ecosystem. (Natarajan, 2002).

The panchagavya is a most fermented product which are used as a plant growth enhancing substances prepared with material available with farmers. They are the rich sources of beneficial micro flora which support, stimulate the plants growth and help in better yield. Formulations which are prepared by using agricultural by-products, which are found to support excellent growth, carrier and storage media for beneficial microorganisms. Panchagavya is known to enhance crop growth and establishment and also sustainable approach in agriculture used by a farmer in south India.

2.0 Importance of Panchagavya:

1. Panchagavya is a liquid organic fertilizer which is useful in organic farming practices.
2. It helps to develop the plants growth through the uptake of plant nutrient and boosts the immunity.

3. Panchagavya improves soil fertility through increasing macronutrients, micronutrients, and beneficial microorganisms, resulting in improved soil health.
4. It increases water holding capacity in soil by acting as organic manure.
5. It promotes the reproduction and growth of beneficial soil microorganisms.

3.0 Ingredients of Panchagavya

- Cow milk-2 litres
- Cow curd -2 litres
- Cow ghee -1 kg
- Cow urine -3litres
- Cow dung -5kg
- Jaggery – 1 Kg in 3 litres of water
- Tender coconut water – 3 litres
- Ripened banana – 12 Nos.

4.0 Method for preparation of panchagavya (Modified from Ram *et al.*, 2018).

In a mud pot the cow dung was mixed thoroughly with ghee and kept for 3 days with a cloth covered the wide mouth of the pot to provide some air. After that this mixture was added with cow urine and water which was kept for 12 days with a couple of mixing in a day using a sterile stick to avoid contamination. Then, the mixture was added with the milk, curd, jaggery, tender coconut water and banana. This mixture is also mixed thoroughly twice a day. At the 30^{th} day the completely fermented material now is known as "Panchagavya".

5.0 Modes of application of panchagavya

The panchagavya can be given to the crops in different ways as per required:

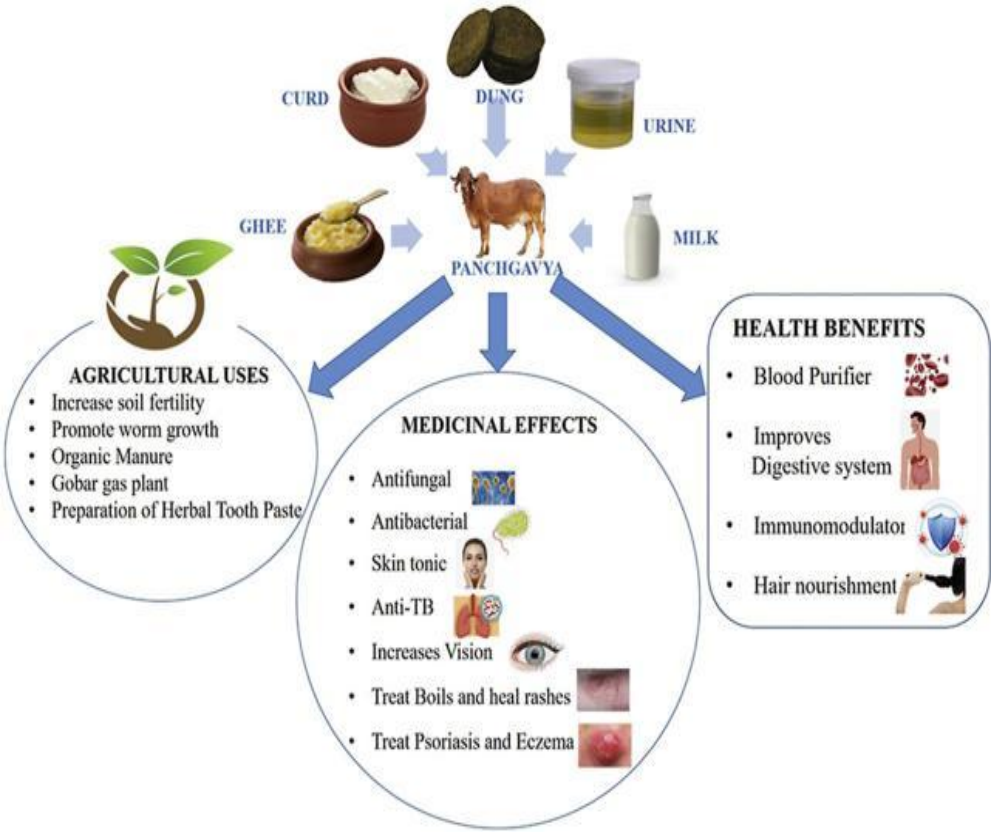
- 5% can be made by mixing the solution of panchagavya(500ml) in 10 litres of water and shake it well, the 5% solution was sprayed in each crop by sprinkler system.
- 50 litres of panchagavya solution per hectare can be given by Microirrigation system or with irrigation water.

- Seeds / seedling: before sowing seeds, the seeds are soaked in 5% of panchagavya solution for 15-20 minutes. The nodules of ginger, turmeric, tuber etc. and the eye piece of sugarcane for 30 minutes soaked in panchagavya solution.

Likewise, the panchagavya is used to treat the small ruminants to get-rid of gastrointestinal worm infections.

In poultry the preparation of panchagavya added with cold rice water gives good immunity to the chicks, improves the layers to yield good quality eggs with high yield at 3% of panchagavya.

6.0 Beneficial effects of panchagavya



(Adopted from Bajaj *et al.*, 2022).

6.1 Microbial activity and soil fertility

Naturally panchagavya contains numerous microbes (Table 1) which are beneficial for plant growth, such as bacteria, fungi, actinomycetes, and some photosynthetic bacteria. Different types of microbial populations are present in panchagavya.

Table 1 Microbial load in Panchagavya (Pathak *et al.*, 2013)

S.No.	**Microorganisms**	**(cfu/ml)**
1	Fungi	3.88×10^3
2	Bacteria	1.88×10^6
3	*Lactobacillus*	2.26×10^5
4	Anaerobes	1.0×10^3
5	Acid formers 360	360
6	Methanogens 250	250

Microbes isolated from panchagavya showed antagonistic activity against *Macrophoimina sp., Alternaria sp.* and *Rhizoctonia solani* (Maiyappan et al., 2016). Dinesh Kumar *et al.*, (2023) suggested that the incorporation of panchagavya spray might enchance the fodder productivity, nutrient accumulation and profitability in the Indo-Gangetic Plain Region (IGPR) in India with reference to maize production.

Application of panchagavya at 3% showed that there is an increase in plant height by nearly 25 %, seed weight, number of seeds per pod, more branches and maximum flowering when compared to control treatments in blackgram variety CO 6 (Gunasekar *et al.*, 2018).

Srimathi *et al.*, 201 3reported that*Jatropha curcas* and *Pongamia pinnata* seed treatment with 5%panchagavya more prevalent than the control and other fixations as far as germination and seedling life for *Jatropha curcas* and *Pongamia pinnata* separately.

Panchagavya at 30 days of fermentation has better of chemical and microbial composition favourable for utilization as a growth promoter and this panchagavya did not has direct antibacterial activity.Mathivanan *et al.*, (2006).

7.0 Role of panchagavya in agriculture

The application of panchagavya in agricultural field demonstrates of several uses:

7.1 Effect on soil:

Panchagavya develops the soil fertility through organic matters, macro and micronutrients levels and uptake of nutrients in plants, promoting the growth and reproduction of microbes that maintaining good soil fertility. It regulates the pH and soil nutrients. In this study the application of panchagavya in agricultural fields the growth and crop yielding by beneficial soil microorganisms around the roots.

7.2 Effect on plants and crops

The panchagavya spraying gives good results in leaves and pests and also enhances photosynthetic materials, which results in maximum yielding. It also develops side shoots from the trunk carrying the maximum numbers of fruits to maturity; rich and high branching. Dense in roots growth in deeper soil layers. It intake of nutrients and water.

7.3 Role in organic farming

Panchagavya helps in the pesticides-free production of food. It also maintains and retain crop production levels when the field changes in inorganic to organic farming practices within a year. It develops the self-life, taste of fruits, grains, and vegetables and yields better and safe quality food products and it improves crop harvest 15days and reduces crop production costs that expenses on chemicals, thereby increasing the profit to the farmer.

8.0 Conclusion

Panchagavya is considered as a wonderful supplement which contains useful miniature organic entities like microorganisms. In crops creation the utilization of eco-accommodating items like panchagavya which are effectively acting on soil as well plants and moreover they are biodegradable. So it is important to utilize common items like panchagavya to create eco-friendly atmosphere with crops and chemicals free food crops and thus panchagavya can play a significant part in natural organic farming practices.

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Streptococcus mutans and its alarming travel from Oral mucosa to Cardiac valves

Jennifer Princy R
Lecturer, Department of Microbiology,
Meenakshi Ammal dental College, Maduravoyal.

Abstract

Oral micro flora habituating inside the human oral cavity gets attached to the tooth surface and tends to develop biofilms. Especially oral microorganisms like *Streptococcus mutans* generally form pathogenic biofilms with the help of intercellular interactions and communications like quorum sensing which remains as the main keys for their endurance (Krzyściak et al 2014). Initially these bacteria gain access to the tooth surfaces and develop dental plaque mediated through biofilm, which further proceeds to dental caries. *Streptococcus mutans* has been implicated as the principle agent responsible for dental caries. They colonize the tooth surface and utilize the sugar source thereby fermenting the carbohydrates producing acid and cause damage to the hard tooth structure leading to dental caries. When the infections become severe, during invasive treatment procedures like dental manipulations, these oral flora can get swept off in the blood to the cardiac valves causing a life threatening condition called Infective endocarditis.

This paper reviews the general features of the bacterium *S.mutans* and its association in disease conditions like dental plaque, dental caries and endocarditis

Introduction

The human mouth with its diverse niche and abundant supply of nutrients is open to different community of bacteria, fungi and other microorganisms, together called as the oral microbiome. The oral microbiome is composed of hundreds of different species of bacteria like Viridans Group Streptococci, *Actinomyces spp, Lactobacillus acidophilus, L. salivarius* (Dewhirst FE et al 2010).

Though these oral commensals prevents the invasion of pathogenic bacteria by competing with them, an imbalance in the oral microbiome occurs due to poor diet, smoking, alcohol consumption and with certain medications thereby altering the healthy environment and results in oral dysbiosis,

proceeding to oral infections. Among them Viridans group Streptoccoci alone form the biggest challenge in the oral cavity.Viridans group Streptococci is the one of the major commensal flora predominating the oral cavity and it includes multiple species and strains of Streptococci like *S.mitis, S.sanguinis, S.mutans, S. salivarius, S. sanguinis* and others. Among them, *Streptococcus mutans* is most commonly addressed for their cariogenic role in dental caries [El Sherbiny GM 2014] as they colonize the teeth surfaces and form dental plaque leading to caries and other serious conditions like Infective Endocarditis [Todar K 2015].

Dental caries remains the most common infectious disease worldwide and, despite being largely preventable, presents a significant global public health problem (Kassebaum et al 2017). Mutans streptococci (MS) like *Streptococcus mutans* and *Streptococcus sobrinus* have been implicated as cariogenic bacteria causing dental infection (Mattos-Graner et al 2014). Dental caries results from a shift in the balance of oral microbiota, with the oral flora being held responsible for caries (Rajendran R, Sivapathasundaram B (2014). A well maintained oral hygiene by mechanical cleaning techniques like brushing and flossing with the use of fluorides, antimicrobial agents, probiotics and a proper dietary intake with reduced quantity of carbohydrates, sugar substitutes is necessary to keep these cariogenic bacteria under control.

Hence this paper which reviews about the pathogenic role of *Streptococcus mutans* in dental caries and Infective endocarditis is discussed.

Streptococcus mutans

Streptococcus mutans is a facultative anaerobic, non- motile, non-spore forming, Gram-positive cocci which appears as short chains under Gram staining and it exhibits alpha hemolysis on blood agar. Mutans streptococci are best identified on selective media, such as Sucrose Bacitracin agar(SB20**),** Mitis-salivarius agar (Hirasawa et al 2003), where they develop deep convex, undulated, adherent colonies with granular frosted glass appearance and small white adherent colonies with irregular margins on Tryptone-yeast extract cysteine with sucrose and bacitracin (TYCSB) agar. They are highly acidogenic (acid producing) and aciduric (acid tolerance) eroding the tooth enamel causing decay and caries. One of their major virulence factor is production of Lipo-teichoic acid which assists in their adherence to the external enamel enabling colonization of tooth surface. Then they utilize the sugar source forming acids, decreasing the pH resulting in demineralization of the enamel surface thereby proceeding to dental caries. During dental interventions and other routine oral activities, these bacteria gain access in to the blood

stream and gets adhered with damaged cardiac valves initiating the commencement of Infective Endocarditis.

They have four serotypes designated as c, e, f, and k, based on the chemical composition of the serotype-specific rhamnoseglucose polymers. Glucosyltransferases (GTFs), protein antigen (PA), and glucan-binding proteins (Gbps) are major surface protein antigens of *S. mutans*. Four types of Gbps (GbpA/GbpB/GbpC/GbpD) of *S.mutans* are also regarded as virulence factors for dental caries, due to their glucan-binding properties [Villhauer, A. L 2017] [Shah DS, Russell RR. 2004].

Streptococcus mutans in Biofilm formation

Streptococcus mutans utilize the sugar source from the dietary carbohydrate intake and in a mutual association with other oral microorganisms they adhere on solid tissues in an extracellular polysaccharide matrix resulting in an oral biofilm. These virulent biofilms includes coaggregation and coadherence of oral bacteria. The Sequential steps leading to the formation of biofilm are (1) Salivary pellicle formation, (2) Initial Adhesion, (3) Maturation of Biofilm by coadhesion of microbial colonizers (5) Dispersion of microbes from mature biofilm (Hannig M (2005)

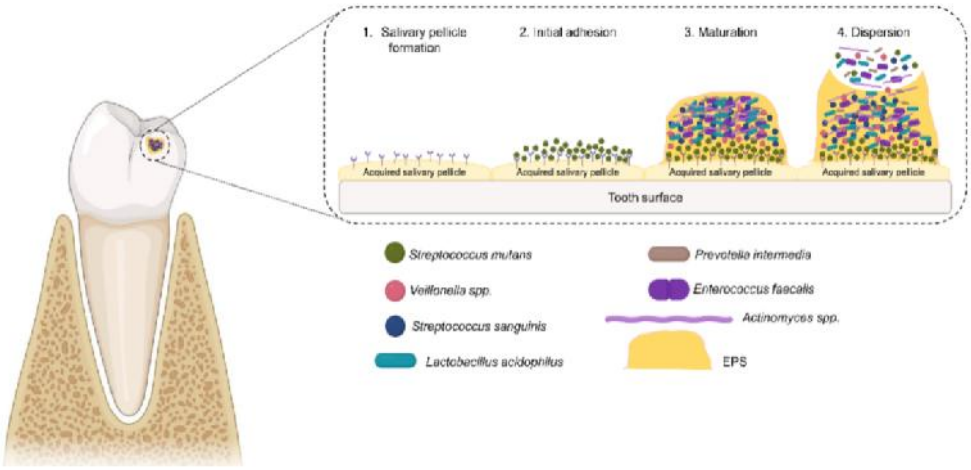

Fig 1: Stages involved in biofilm formation (Adapted from Milho, C et al (2021).

The microbiological study on dental plaque dates back to 1924 when Clarke first observed oral Streptococci (Clarke JK1924). Although an oral commensal, *Streptococcus mutans* has been marked as long implicated potential pathogen associated with dental caries due to the formation of

virulent dental biofilms (Wang et al 2020). Alteration in the homeostasis of the oral cavity along with an increased density of cariogenic bacteria is recognized as the primary cause of the disease. As the primary etiological agent, *S. mutans* has developed multiple mechanisms to integrate into the dental biofilm (plaque) to colonize tooth surfaces. The significant factors associated with cariogenicity include adhesion, acidogenicity, and aciduricity. One of the prominent pathways is initiated when *S.mutans* produces glucans, via glucosyltransferases (Gtfs) to catalyze the synthesis of extracellular polysaccharides (EPS). The synthesized glucan allows *S.mutans* to effectively colonize on the tooth surfaces and favors its adherence with other oral microorganisms, food debris, and salivary components to create a cariogenic biofilm environment (Zhu W, et al 2017), that increases protection against mechanical host-clearance forces and different antimicrobial agents (Ito et al (2020)

S. mutans possesses three Gtfs, which are products of gtfB, gtfC, and gtfD genes; GtfB synthesizes mostly insoluble glucans containing more of α-1,3-linked glucans and GtfC synthesizes a mixture of soluble and insoluble glucans, whereas GtfD synthesizes predominantly soluble glucans containing more α-1,6-linked glucans. (Xiao J et al (2010). Additionally, they have the ability to ferment most of the sugars present in the food and produce insoluble extracellular polysaccharides, which favors in its permanent adherence to the tooth surface and encourage biofilm formation (Oliveiraet al., 2022). In addition, *S. mutans* also produces mutacins (bacteriocins) which is important factor in colonization of *S. mutans* in dental bioflm (Banu 2010; Matsumoto-Nakano 2018; Krzyściak et al. 2014).

Streptococcus mutans in Dental caries

Dental caries is a multi-factorial infectious disease associated with demineralization and progressive destruction of the tooth enamel mediated by bacteria and their virulence factors leading to loss of tooth(Bowen et al.,2018). Dental caries occurs primarily during increased consumption of carbohydrate diet which alters the homeostasis of oral microbiome with acidophilic bacteria like *Streptococcus mutans* [Simon L (2007)]. Dental caries is a chronic childhood disease which tends to occur soon after the eruption of first teeth, and may end either as a painful cavities which needs dental interventions and even sometimes becomes severe requiring hospitalization [Gross EL, et al (2012)]. Hence, considered as an important event in the loss of tooth. *Streptococcus mutans* and *Streptococcus sobrinus* are the two most common Mutans Streptococci isolated from dental caries

Change in life habits with intake of readily available sugars in packaged drinks and food with improper maintenance of oral hygiene depict the initial phases of caries (Nishimura et al 2012).

Various factors that contribute to a person's risk of acquiring dental caries includes environmental aspects like malnourishment, improper oral hygiene, risk of fluoride, and the degree of colonization of cariogenic bacteria; and host factors being salivary discharge, salivary buffering capacity, position of teeth connected to one another, surface features of tooth enamel, and size of occlusal fissures present on posterior teeth. Furthermore, the hereditary changes of the host can also extend the threat more towards caries [Marsh PD 2012]

All these potential virulent mechanisms subsequently calls out for dental interventions like root canal treatment and extraction. And now during conditions like dental manipulations, oral infection and with low dental hygiene, these cariogenic pathogen gains entry into the blood stream which leads to transient bacteremia and cause infective endocarditis.

As a precautionary measure, antibiotics are being prescribed by the dentists before treatment, inorder to prevent any systemic infections arising after cavity filling or tooth extraction. But as of now with proceeding adverse reactions of antibiotics and with increase in the rise of multidrug-resistant bacteria, decreased susceptibility to antimicrobials has become a great threat to the health of the community [Salman, H. A 2017]. Hence it is necessary to screen for the antimicrobial susceptibility of these oral cariogenic bacteria like *Streptococcus mutans.*

Initially β-lactams are the drug of choice for treating dental caries but there has been increased resistance of penicillin among mutans Streptococci which has further complicated the therapy of infective endocarditis. With increase in the rate of infection, the treatment progress with a variety of antibiotics, including penicillin, erythromycin, clindamycin, tetracycline & chloramphenicol, leading to the rapid spread of resistance. Periodic surveillance of antibiotic susceptibility among these cariogenic bacteria has to be carried out by clinical microbiology laboratories worldwide.

Streptococcus in Infective Endocarditis

Infective endocarditis is a rare but usually severe and often fatal inflammatory disease affecting the native and prosthetic valves and cardiac tissues. The incidence of cardiovascular diseases associated with oral bacteria has currently gained the center of consideration. It is believed that the

cariogenic flora, *Streptococcus mutans is* known to play a crucial role in the development of endocarditis, especially in patients with underlying cardiac conditions, such as valve replacement, congenital heart disease and a history of previous infective endocarditis. Endocarditis is a life threatening disease with a mortality of up to 50% inspite of antibiotic treatment. Presence of endocarditic plaque constituting bacteria, platelets, coagulation factors and leucocytes on the leaflets of one or more heart valves depicts the characteristic feature of endocarditis (Donlan R.M et al (2002). Cytotoxic chemotherapy causes severe mucositis enabling the oral flora to be carried away in to the bloodstream through the damaged mucosa causing sepsis and bacteremia and eventually they colonize the damaged heart valve that produces vegetations. Complications are septic shock and the adult respiratory distress syndrome (Marron, A et al (2000).

The ability to form biofilms may be a prerequisite for the pathogenesis of endocarditis. The biofilm formed by the oral microbes along with cariogenic, mutans streptococci, under certain conditions may extend their arm in to the cardiac tissues leading to carditis, reducing the treatment options leading to complications. Antimicrobial prophylaxis prior to dental interventions was initially recommended to prevent this mechanism. Recently, numerous medical professionals worldwide have published guidelines and updates on the prevention of endocarditis with divergent recommendations regarding the requirement of prophylactic antibiotic regimen prior to dental procedures. Recent evidences supports that Infective endocarditis caused more likely by transient bacteremia from an oral source results from daily routine activities rather than a dental procedure (Baddour et al. 2015; Habib G, 2015 Thornhill MH, et al. 2016). Therefore properly maintained oral health with regular dental care is important to prevent IE caused by *Streptococcus mutans* (Garg et al 2019).

Laboratory Diagnosis:

The samples chosen for isolation of S. *mutans* include gentle swabbing over the surface of caries, dental plaque obtained using the toothpick technique or paper points and whole saliva samples being collected in sterile glass container [El Sherbiny GM (2014). The samples were then transferred to the laboratory for further procedures like Culturing, Isolation and Detection. Identification of *Streptococcus mutans* from other streptococci was done according to the Following procedure.

a. Gram staining with its characteristic appearance as Gram positive cocci in short chains

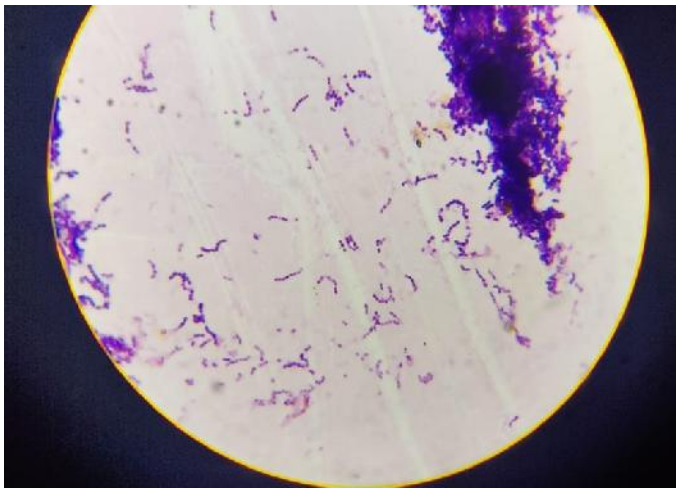

Figure No 2: Gram staining of Streptococcus mutans isolated from dental plaque

a. Blood agar with alpha hemolysis

b. Colony morphology on selective medium like Mutans Sanguis agar, Mitis-Salivarius bacitracin agar, TYCSB agar (tiny white colonies with clear zone) thereby incubating anaerobically for 24 hrs at 37°C [Mohapatra SB (2012)]

Fig 3: *S. mutans* on Mutans Sanguis agar: Convex undulated colonies of white color with rough margins, characteristic granular frosted glass appearance and adherent to the agar

c. Biochemical characterization was done to identify the species as described by Fngold and Baron (1986). *S.mutans* was identified by fermentation of melibiose and raffinose, but not of mannitol and inulin, and ability to release ammonia from arginine and hydrolyse esculin. (Villhauer, A et al 2017)

e.Dextrane production test [Guthof (1970)]

Genotypic Identification of *Streptococcus mutans*

Genetic approaches such as a species-specific PCR based on the dextranase or glucosyltransferase gene sequences, especially gtfB of *S. mutans* and gtfI of *S. sobrinus* have been reported to be useful for detecting *Streptococcus mutans* and *Streptococcus sobrinus* (Oho et al 2000). In addition to these target genes, bacterial 16S rRNA gene is known to be highly conserved within bacterial species and is widely used as a tool of bacterial taxonomy (Igarashi, T et al 2000).

Detection of *S.mutans* by PCR amplification is rapid and shows clear identification of species. Nested PCR is able to identify *S. mutans* rapidly and directly from human whole saliva. This achievement is proved to be very valuable to analyze and explain the role played by the streptococcal species in the etiology of dental caries (Tian HP, et al 2003). Recent studies reported the successful development of a LAMP-based microfluidic system for the rapid and quantitative detection of *S. mutans* (Wang et al 2023)

Management of the Diseases:

Treatment:

The acceptable treatment options for dental caries is to get filled the cavity by dentists and to enlighten adults regarding appropriate oral hygiene and nutritional habits [Simon L (2007). *S. mutans* which develops inside dental plaque exhibits a considerable decrease in susceptibility to antibiotics and antimicrobial agents than the antimicrobials employed in toothpastes and mouthwashes like chlorhexidine, which provides broad spectrum antibacterial, antiviral and antifungal activity, and is considered to have antiplaque activity too.

Prevention:

Preventive measures primarily aims at removal of biofilm- induced caries so that the related dental problems and invasive conditions can be kept under control [Metwalli KH et al (2013) favorably by enhancing the attribution

of oral hygiene [Longbottom C (2009)]. Oral care, certainly followed by oral hygiene helps in clearing away the bacteria and food materials from mouth.

Following approaches have been recommended for the prevention of caries:

a. Mechanical cleansing techniques with regular brushing and flossing of teeth benefit the humans to prevent dental caries.

b. Use of fluoridated tooth paste and mouth washes.

c. Reduced consumption of glucose which cuts down the cariogenic bacteria and by using xylitol, sorbitol, lycasin which has anticariogenic activity

d. Using antimicrobial agents like Vancomycin, penicillin and erythromycin has also shown extensive results and by using Chlorhexidine and Sodium hypochlorite in mouth washes.

e. Passive Immunization by using monoclonal antibodies against antigens of mutans streptococci prevent recolonization by the organisms (Samaranayake L (2007)

f. Probiotics: Probiotic bacteria are employed to inhibit the oral bacteria which are involved in dental diseases [Marsh PD (2012]

g. Dairy products (milk and milk products): Cheese has been proved to raise salivary flow rates and to promptly promote plaque pH shifts trailing to wash off sucrose.

All these advancements have increased the surge for new caries preventive procedures in the near future.

Conclusion

An awareness among the general public to take correct actions to minimize the risk for the dental disease should be framed. Though, the effects of this bacteria are severe dental caries, dental plaque and endocarditis, it can be easily eliminated by increasing the oral hygiene, and control in intake of carbohydrate diet. Hence, inorder to control these infections, it is required to take necessary precautions in plaque control, including brushing twice a day, flossing, and professional scaling, reduction in sucrose rich foods, regular mouth washing. Antimicrobial resistance among VGS alarms a great threat to clinicians as it creates an obstacle to antibiotic therapy, in case of endocarditis (Rotimi, 2005). It also creates a serious clinical problem in the management of therapy (Amrouche *et al*., 2004).

VGS also acts as a reservoir of antimicrobials resistant genes and they exchange resistance determinants to closely related and more pathogenic bacterial species like *Streptococcus pyogenes* and *Streptococcus pneumonia*. This varied range of exchange of resistance among viridians group streptococci alarms us to continuosly update its susceptibility for a wide range of antibiotics which would further enhance the treatment strategies & ensure the provision of safe and effective empiric therapies.

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Removal of Microplastics from the Environment: A Review of Current Technologies

[1*]Sona A, [2]Priya Senan V, [3]Jyothilekshmi S
[1,2,3] Associate Professor
[1,2]Department of Biotechnology, SAS SNDP Yogam College Konni, Pathanamthitta, Kerala, India
[3] Department of Biosciences, Union Christian College Aluva, Ernakulam, Kerala, India

Abstract

Microplastics are a growing environmental concern due to their potential negative impacts on human health and the environment. There are a number of technologies that have been developed for the removal of microplastics from the environment, including physical, chemical, and biological methods. The choice of the most appropriate method will depend on a number of factors, including the type of microplastic, the concentration of microplastics, and the environmental conditions. The development of new and improved technologies for the removal of microplastics is an active area of research. By continuing to invest in research and development, we can develop the technologies that are needed to tackle this growing environmental challenge.

Key words: Pollution, Marine life, Human health, Recycling, Sustainable practices, Waste management

Introduction

Microplastics are small plastic particles that are less than 5 millimeters in size. They can be found in a variety of environmental media, including air, water, soil, and food. Microplastics can be generated from a variety of sources, including the breakdown of larger plastic items, the intentional use of microbeads in personal care products, and the release of plastic fibers from textiles. Some of the sources are listed below.

Sources of Microplastics

Microplastics can enter the human body through a variety of sources, including:

- Food: Microplastics can be found in a variety of foods, including seafood, bottled water, and processed foods.

- Water: Microplastics can be found in tap water, bottled water, and even rainwater.
- Air: Microplastics can be inhaled through the air, especially in urban areas.
- Soil: Microplastics can be found in soil, especially near industrial areas.
- Personal care products: Some personal care products, such as facial scrubs and toothpaste, contain microbeads.

Microplastic pollution is a growing environmental concern because of the potential negative impacts that microplastics can have on human health and the environment. Microplastics can be ingested by a variety of organisms, including marine animals, birds, and humans. Once ingested, microplastics can accumulate in the body and have a range of adverse effects, including inflammation, oxidative stress, and cancer.

Figure 1: Microplastics in air

Microplastics in the Soil

Microplastics are a ubiquitous environmental pollutant, originating from various sources such as plastic debris fragmentation, industrial processes, and microfiber shedding from textiles. They pose a threat to terrestrial ecosystems, with soil being an essential component of the Earth's environment.

Sources of Microplastics in Soil:

1. **Plastic Pollution:** As plastic waste accumulates in the environment, it undergoes mechanical and chemical degradation, breaking into smaller particles that eventually find their way into soil.

2. **Agricultural Practices:** The use of plastic mulch films, which are extensively employed in agriculture, can degrade into microplastics, which then mix with the soil.
3. **Wastewater Irrigation:** The application of treated wastewater for irrigation in agriculture can introduce microplastics from effluents into the soil.
4. **Microfiber Shedding:** Synthetic textiles release microplasticfibers during washing, which can end up in soil.

Figure 2: Microplastics in soil

Distribution in Soil

Microplastics in soil can be found in various forms, including microbeads, fragments, and fibers. Their distribution within the soil profile depends on factors such as soil type, land use, and proximity to pollution sources. Recent studies have shown that microplastics are not only present on the soil surface but can also be transported to deeper layers through bioturbation and other soil processes.

Impact on Soil and Ecosystems:

1. **Soil Health:** Microplastics can alter soil physical properties, affecting water retention, aeration, and nutrient availability. This can impact plant growth and crop yields.
2. **Ecological Consequences:** Soil-dwelling organisms, such as earthworms and microorganisms, can ingest microplastics, potentially disrupting soil food webs and nutrient cycling.

3. **Contaminant Transport:** Microplastics can adsorb harmful chemicals, including pesticides and heavy metals, potentially facilitating their transport through soil and into groundwater.
4. **Human Health:** Although the direct impact of microplastics in soil on human health is still being studied, the potential for human exposure through crops grown in contaminated soil raises concerns.

Mitigation Strategies:

1. **Plastic Waste Reduction:** Reducing plastic production and promoting recycling can decrease the input of microplastics into the environment.
2. **Alternative Mulch Materials:** Developing biodegradable or recyclable alternatives to plastic mulch films in agriculture.
3. **Improved Wastewater Treatment:** Enhancing wastewater treatment processes to remove microplastics before discharge.
4. **Soil Remediation:** Developing techniques for the removal of microplastics from contaminated soil.

Microplastics in the Marine Environment

The presence of microplastics in the oceans has been a growing concern for many years. These tiny plastic particles can be ingested by marine life, leading to physical damage and potential toxicity effects. They may also leach plastic additives, including persistent organic pollutants (POPs) and potentially toxic elements, which can accumulate in the tissues of organisms and be passed up the food chain.

There is a lot of research being done on the potential effects of microplastics on biota and humans. Some studies have shown that microplastics can cause damage to the digestive system, liver, and other organs. They have also been linked to cancer and other health problems.

However, it is important to note that many of these studies have been done using high concentrations of microplastics that are not typically found in the environment. More research is needed to determine the true risks of microplastics to human health.

Figure 3: Microplastics in the marine environment

Microplastics in Food

Microplastics have emerged as a global environmental issue, but their presence in the food we consume has gained particular attention. These microscopic particles find their way into the food chain through various sources, including environmental contamination, food packaging, and processing. This article discusses the occurrence of microplastics in food and their potential risks to human health.

Sources and Pathways of Microplastics in Food:

1. **Environmental Contamination**: Microplastics enter aquatic environments through runoff, atmospheric deposition, and wastewater discharge, where they are ingested by aquatic organisms. These organisms, including fish and shellfish, can accumulate microplastics in their tissues.
2. **Food Packaging and Processing**: Plastic packaging materials and processing equipment can release microplastics into food. Additionally, the abrasion of plastic packaging during transportation and storage can contribute to microplastic contamination.
3. **Agricultural Practices**: The use of plastic mulch films and sewage sludge as fertilizers can introduce microplastics into agricultural soils. These particles can then be taken up by plants, potentially entering the food supply.

Health Implications of Microplastics in Food:

1. **Ingestion**: Microplastics in seafood and bottled water have been documented, raising concerns about human ingestion. While the long-term health effects are not fully understood, there is a potential risk of gastrointestinal and other health issues.
2. **Chemical Exposure**: Microplastics can adsorb toxic chemicals from the environment. When ingested, these chemicals may leach into the digestive system, potentially exposing consumers to harmful substances.
3. **Microbiome Disruption**: Microplastics in the gastrointestinal tract may affect the gut microbiome, which plays a crucial role in overall health and immunity.
4. **Translocation**: Studies suggest that microplastics may translocate from the gut to other organs, such as the liver and spleen, raising concerns about systemic health effects.

Regulation and Future Research: Regulation and monitoring of microplastics in food are still in their infancy. To address this issue effectively, the following steps are essential:

1. **Standardized Testing Methods**: The development of standardized methods for detecting and quantifying microplastics in food is crucial for consistent monitoring.
2. **Risk Assessment**: In-depth risk assessments are needed to determine the health effects of microplastic ingestion, including potential toxicity and bioaccumulation.
3. **Reducing Plastic Use**: Initiatives to reduce plastic production, improve recycling, and promote sustainable packaging practices can help mitigate the influx of microplastics into the food supply.
4. **Consumer Awareness**: Public education campaigns can raise awareness about microplastic contamination in food and encourage consumers to make informed choices.

Microplastics and Humans

Microplastics can be ingested by humans through food, water, and air. Once ingested, microplastics can accumulate in the body and have a range of adverse effects, including inflammation, oxidative stress, and cancer.

There is still much that we do not know about the environmental and health impacts of microplastics. However, the research that has been done so far suggests that microplastics pose a real threat to human health.

Figure 5: Microplastics in human

Health Effects of Microplastics

The health effects of microplastics are still being studied, but there is some evidence that they can cause a variety of health problems, including:

- **Inflammation:** Microplastics can cause inflammation in the body, which can lead to a variety of health problems, such as heart disease and cancer.
- **Oxidative stress:** Microplastics can cause oxidative stress, which can damage cells and DNA.
- **Cancer:** Some studies have shown that microplastics can cause cancer in animals.
- **Neurological disorders:** Microplastics have been linked to neurological disorders, such as Alzheimer's disease and Parkinson's disease.
- **Reproductive problems:** Microplastics have been linked to reproductive problems, such as infertility and miscarriage.

Risk Assessment

The risk of microplastics to human health is difficult to assess, as there is still limited data on their toxicity. However, the available evidence suggests that microplastics pose a potential risk to human health.

Mitigation Strategies

There are a number of strategies that can be used to mitigate the risks of microplastics to human health, including:

- **Reducing plastic production:** Reducing the production of plastic is the most effective way to reduce the amount of microplastics entering the environment.
- **Improving waste management:** Improving waste management can help to reduce the amount of plastic that ends up in the environment.
- **Developing biodegradable plastics:** Developing biodegradable plastics can help to reduce the amount of plastic that persists in the environment.
- **Educating the public:** Educating the public about the risks of microplastics can help to reduce the demand for plastic products.

Steps to reduce the amount of microplastics entering the environment:

- Reducing the use of single-use plastics
- Recycling plastics properly
- Avoiding products that contain microbeads
- Choosing sustainable clothing that is made from natural materials

Methods for Removing Microplastics:

There are a number of technologies that have been developed for the removal of microplastics from the environment. These technologies can be broadly classified into physical, chemical, and biological methods.

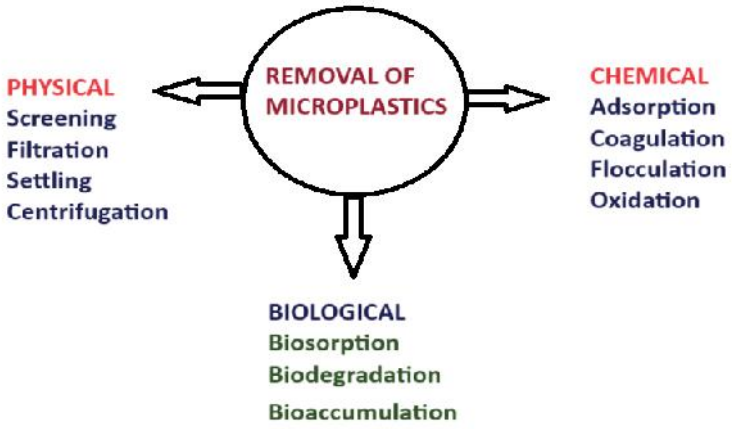

Figure 6: Microplastic Removal Methods

Physical Methods

Physical methods for the removal of microplastics from the environment rely on the physical properties of microplastics, such as their size, shape, and density. These methods include:

- **Screening:** This method uses screens to physically remove microplastics from water or air. The screens have different mesh sizes, which allow the passage of smaller particles while trapping larger particles.
- **Filtration:** This method uses filters to remove microplastics from water or air. The filters can be made of a variety of materials, such as cellulose, glass fiber, or activated carbon.
- **Settling:** This method relies on the gravitational force to remove microplastics from water. Microplastics will settle to the bottom of a container over time, where they can be collected.
- **Centrifugation:** This method uses centrifugal force to remove microplastics from water. Microplastics will be concentrated at the bottom of the centrifuge tube after centrifugation.

Chemical Methods

Chemical methods for the removal of microplastics from the environment rely on the chemical properties of microplastics. These methods include:

- **Adsorption:** This method uses a substance to attract and trap microplastics. The substance can be a natural material, such as activated carbon, or a synthetic material, such as polystyrene.
- **Coagulation:** This method uses chemicals to clump together microplastics. The clumped microplastics can then be more easily removed from the water.
- **Flocculation:** This method uses chemicals to cause microplastics to form larger particles. These larger particles can then be more easily removed from the water.
- **Oxidation:** This method uses chemicals to break down microplastics into smaller molecules. The smaller molecules are then more easily removed from the water.

Biological Methods

Biological methods for the removal of microplastics from the environment rely on the use of living organisms. These methods include:

- **Biosorption:** This method uses microorganisms to absorb microplastics. The microorganisms can then be removed from the water and the microplastics can be recovered.
- **Biodegradation:** This method uses microorganisms to break down microplastics into smaller molecules. The smaller molecules are then more easily removed from the water.
- **Bioaccumulation:** This method uses organisms to accumulate microplastics in their tissues. The organisms can then be harvested and the microplastics can be removed from their tissues.

The choice of the most appropriate method for the removal of microplastics from the environment will depend on a number of factors, including the type of microplastic, the concentration of microplastics, and the environmental conditions.

Conclusion

The removal of microplastics from the environment is a challenging task, but it is essential to protect human health and the environment. The development of new and improved technologies for the removal of microplastics is an active area of research. By continuing to invest in research and development, we can develop the technologies that are needed to tackle this growing environmental challenge.

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Nanotechnology in Medicine

Divyasree.A[1], Renuka Devi.K[2] and Deepa Rajendiran[3*]

[1, 2]First Year BDS, Department of Biochemistry, Madha Dental College and Hospital, Chennai, Tamil Nadu, India.

[3*]Associate Professor, Department of Biochemistry, Madha Dental College, and Hospital, Kundrathur, Chennai, Tamil Nadu,India.

Corresponding Author: Dr. Deepa Rajendiran M.Sc., M.Phil., Ph.D. Associate Professor, Department of Biochemistry, Madha Dental College and Hospital Kundrathur, Chennai, Tamil Nadu, India.

E-Mail: *deepanish1981@gmail.com*

Abstract

Nanotechnology, a very broad area of research that has great potential in many fields, including health, construction, and electronics, involves manipulating material at the atomic or molecular level to create materials that exhibit astonishing heterogeneity and new properties.In medicine, it is used for the delivery of drugs, gene therapy, diagnosis and the complete transformation of other research, development and medical applications.

Keywords: Nanotechnology, Medicine, Nanoparticles, Cancer

1. Introduction

The concept of Nanotechnology was first introduced in 1959 when physicist Richard Feynman presented a presentation on making things at the atomic and molecular levels [1]. It is the treatment of individual atoms, molecules or compounds into structures to produce materials and devices with special properties. It deals with materials in the size of 0.1-100nm [2]. The ability to manipulate structures at atomic scale allows for the creation of nanomaterials. Nanotechnologies have had a significant impact in almost all industries and areas of society as it offers better built, safer and cleaner. Nanomaterial allows mass-creation of product with enhanced functionality, significantly lower costs, greener and cleaner manufacturing processes, to improve healthcare and reduce the impact of manufacturing on the environment [3].

Nanomedicine uses technologies at nanoscale and Nano-enabled techniques to prevent, diagnose, monitor and treat disease. Nanotechnologies exhibit significant potential in the field of medicine, including in imaging techniques and diagnostic tools, drug delivery systems, tissue-engineered constructs, implants and pharmaceuticals therapeutics [3]. A benefit of using nanoscale for medical technologies is that smaller devices are less invasive and possibly be implanted inside the body, plus biochemical reaction times are much shorter[4]. The Nanomaterials are having interesting optical, magnetic and electrical properties which are having significant effects in the fields of electronic medicine. Nanomedicine's primary fields of use include:

- Delivery of pharmaceuticals
- *In vitro* and *in vivo* diagnostic, including imaging
- Regenerative medicine
- Implanted devices

2.Nanoparticles

Nanoparticles (NPs) are technically defined as particles with dimensions less than 100 nm and unique properties not typically found in bulk samples of the same material [5]. Nanoparticles are gaining importance in various field because of their exceptional features like high surface, volumetric ratio, dissimilarity, submicron size, and improved targeting systems.NPs can have different shapes, sizes, and structures [6].They can be spherical, cylindrical, conical, tubular, hollow core, helical, etc. NPs were found to penetrate deep into tissues and enhance the permeability and retention (EPR) enhancement effect. Additionally, surface properties influence bioavailability and half-life by effectively penetrating epithelial fenestrations [7].

NPs can be homogeneous or composed of multiple layers. In the latter case, the layers would look like this: (a) The surface layer is usually composed of various small molecules, metal ions, surfactants, or polymers. (b) A cladding layer consisting of a chemically different material from the core layer. (c) Nuclear layer representing the central part of the NP [8]. Diagnostic and therapeutic nanoparticles are generally fall into two categories: a) Organic nanoparticle (liposome and micelles) b) Inorganic nanoparticles (Silica, gold, iron oxide, etc).

3.Types of Nanoparticles

Some common types of nanoparticles are discussed below

❖ Micelles

Micelles spontaneously aggregate and self-assemble into spherical vesicles under aqueous condition with hydrophilic outer monolayer and hydrophobic core. Recently, micelles loaded with metal nanoparticles (MNPs) have been used in several biological applications due to their biocompatibility, pharmacokinetics, adhesion to biological surfaces, targeting, and longevity.The size of micelles ranges from 10-100 nm [9].

❖ Liposomes

Liposome, the old version of lipid nano particle sizes ranging from 30nm to several microns, that consist of lipid bilayer. They can transport hydrophilic or hydrophobic molecules such as proteins, nucleic acids and small molecules.Several liposomal drug formulations have been approved and successfully applied to medical practice [3].

❖ Dendrimers

Dendrimer are nano molecules with regular branching structure. The branches arise from the core in shape of a spherical structure by means of polymerisation. It contains three different regions: core moiety, branching units and closely packed surface. Dendrimers can be employed in place of traditional viral vectors in gene therapy. The number of branching determines the size of dendrimers which can be controlled. It sizes less than 10 nm. Conjugates of dendrimers with saccharides or peptides have been shown to exhibit enhanced antimicrobial and antiviral properties with improved solubility and stability upon absorption of therapeutic drug [10].

❖ Quantum dots

Quantum Dots (QDs) are nanocrystals of size 2-10 nanometers that can be converted into fluorescence when stimulated by light [6]. Semiconductor particles having optical and electronic properties that differ from those of larger particles as a result of quantum mechanics [8]. QDs have been successfully demonstrated due to their unique properties, including outstanding photostability, size-dependent optical properties, high extinction and brightness coefficients etc. Quantum dots provide a versatile tool to support more accurate diagnostics and immunofluorescence assays, multichannel imaging and therapeutic platforms, imaging of cellular and in vivo processes in real time and track single cells and biomolecules [11].

❖ Metallic nanoparticles

Metal nanoparticle are flexible nanostructures due to their ability to control shape, composition, size, structure, assembly, and optical properties [12]. They are generally solid colloidal metal particles of size range 10-1000 nm that incorporates a therapeutic molecule which remains either dispersed in the matrix of polymer carrier, entrapped within a polymer shell, or attached or adsorbed by covalent linkage to the surface of the particles. Metallic nanoparticles have been used as imaging contrast agents, in laser-based treatment, as optical biosensors and drug delivery vehicles.Among the various metals, silver and gold nanoparticles are of prime importance for biomedical use[13].

4. Applications of Nanotechnology in Medicine

Nanotechnology products have been increasingly beneficial in healthcare, resulting in the development of unique nano systems for the diagnosis, imaging, and treatment of a variety of diseases, including cancer, cardiovascular, ophthalmic, and central nervous system ailments [14].One of the most important steps in clinical practice is to diagnose a disease. It is used as sensors, analyte detection, pathogen detection and separation, imaging agent, targeted imaging, delivery vehicles[15].Nano systems provide precise drug delivery to target tissues or organs with controlled release and increased retention time over conventional approaches.Nano-liposomes are one of the best examples of nano systems now being developed for targeted medication delivery in the treatment of various malignancies and cardiovascular illnesses [16].

Cancer is a major disease characterized by aberrant cell growth with the ability to invade or spread to other bodily parts, often known as malignant tumors or malignant neoplasms. There are over 100 different known cancers that affect humans, but most common types are breast cancer, lung cancer, colon cancer, prostate cancer [17]. Nanotechnology is important in the development of cancer treatments and the identification of cancer biomarkers. To target malignant tumors with increased specificity and affinity, NPs can be combined with bio-targeting ligands such as small molecules, peptides, and monoclonal antibodies [18].Nanoparticles bound to antibodies via polyethylene glycol are used in the treatment of breast cancer [19].

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in developed and developing countries. Early diagnosis of coronary artery disease increases the chance of successful treatment and possible cure,

giving patients a better prognosis and longer survival. Mass spectrometry is commonly used to identify potential biomarkers for Coronary Artery Diseases, but is limited in sensitivity and specificity due to low biomarker concentrations in human plasma.For these reasons, the combination of nanotechnology with biosensors may constitute a promising solution for diagnosing coronary artery disease in its early stages[20].

Recent advances in nanotechnology have significantly benefited tissue engineering, which is used to repair or regenerate damaged tissues or organs and to design smart drug delivery systems.eg: Titanium based materials have been applied in bone tissue engineering [21].The findings of nanomaterial research have been published based on studies conducted on the following tissues: bone, cartilage, nervous system, skin and heart muscle [22].The materials used include carbon nanotubes (CNTs). Their unique properties have opened up the possibility of the use of CNTs in therapies that focus on repairing damaged tissues, especially those requiring electrical stimulation [23]

Over the past few decades, different nanomedicines have been approved for clinical use from the Food and Drug Administration (FDA). Among the approved nanomedicines, liposomal, polymeric, and micelles were represented and administered using oral, intravenous, and transdermal pathways.Nanodrugs were authorised for a wide range of indications, including cancer[24].Nanotechnologies have also led to extensive discussions on their safety and any health risks associated with their use, although the emerging field of nanotechnology has attracted a great deal of public interest.The use of nanomaterials presents new challenges, notably the identification, evaluation and management of potential health risks[25].

5. Conclusion

Nanotechnology has emerged as a new exciting field in science, with several potential applications for medicine. With a series of innovations and advances in nanomedicine technology, diagnosis and treatment at the micro-level is increasing, and nanotechnology is widely used in medical treatment, diagnosis, and other medical fields.

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Microbes an alternative source of polyunsaturated fatty acids: Towards high value ingredients for feed and pharmaceuticals

Anuradha Jape
Assistant Professor in Microbiology, Y.M. College of Arts, Science and Commerce, Bharati Vidyapeeth, Erandwana Campus, Pune411038
E-mail: *anuradha.jape@gmail.com*

Devyani Salunke
Microbiologist, Centre for Food Testing, Bharati Vidyapeeth, Pune
E-mail: *devyanipsalunke@gmail.com*

Abhay Harsulkar
Professor, Poona College of Pharmacy, Bharati Vidyapeeth, Pune
E-mail: *aharsulkar@yahoo.com*

Contents:

Introduction

About 60 years ago a series of epidemiological studies were published that elaborated lower risk of coronary heart disease and diabetes in Greenland Eskimos and in Japanese from Kolhama Islands who consumed more omega 3 PUFAs from marine fish, whales, and seals. In the Eskimo population, dietary intake primarily consists of long-chain fatty acids, notably Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA), while linoleic and linolenic acids are present in lower quantities. This dietary pattern contributes to reduced

levels of serum triglycerides and very low-density lipoproteins (VLDL) in Eskimos compared to the Western population. Fatty acids are categorized as saturated or unsaturated, depending on the presence and location of double bonds, which can lead to monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). While humans can synthesize certain fatty acids, there are essential fatty acids that must be acquired through dietary sources.

1. LCPUFA in health: An overview:

Since ancient times, cod liver oil has been regarded to confer beneficial effects on human health and used as a supplement to boostcardiac health. In 1965 Fatty acid composition of cod liver oil was characterized by Lambertsen and Brkkan. In the past five decades, severalstudies on LCPUFA have highlighted the beneficial role of omega3 and omega 6 fatty acids in normal development and well-being. Polyunsaturated Fatty Acids (PUFA) are essential components in higher animals. Being an important component of cell membrane PUFA play crucial role in maintaining membrane fluidity, flexibility, and selective permeability. Additionally, PUFA as regulatory molecules govern various physiological functions like modulation of ion channels, transport processes, endocytosis, exocytosis and functions of membrane-associated enzymes, neurotransmission, *etc.* In mammals, metabolites resulting from LC-PUFA such as prostaglandins, leukotrienes and thromboxanes regulate various cellular functions. Responses to signals, development and maintaining physiological process at various growth phases, maintaining mental health, protection against cardiovascular illness, prevention of cancer, *etc.* are regulated by EFAs. Fatty acids act synergistically and act as preventive or therapeutic agent in the treatment of various disorders like hypertension, dyslipidemia, type-2 diabetes, obesity, metabolic syndrome,etc.

2. Nutrition: Importance of the ratio of omega 6 and omega 3 essential Fatty Acids

The ideal dietary ratio of n-6 to omega-3 fatty acids is approximately 1:1, which is vital for maintaining homeostasis and supporting normal development. Unfortunately, this balance has shifted to an alarming 20:1 due to the influences of modern agriculture and changing dietary habits. In recent years, there has been a notable increase in meat consumption, contributing to this skewed ratio.The occurrence of metabolic disorders in the population has significantly increased primarily due to is increased intake of high saturated fats and low intake of EFAs. Disproportion in the dietary fatty acids affects the dynamics, phase changes and fluidity, permeability of membrane and exert

metabolic disturbances.The interplay of polyunsaturated fatty acids in cellular physiology and their critical role in maintaining good health are well understood. Increased intake of omega 6 fatty-acid with corresponding decrease in omega 3 fatty-acid in diet has led to chronic conditions like cardiovascular disease, diabetes, cancer, obesity, autoimmune diseases, rheumatoid arthritis, asthma and depression (FAO report 2008). Various international scientific authorities, Institute of Medicine (IOM), European Food Safety Authority (EFSA) have published recommendations for daily intake of (omega 3) PUFA and has prescribed 250 mg of EPA plus DHA for adults and additional 100–200 mg DHA during pregnancy and lactation. Commercial PUFA rich preparations have applications in biomedical and nutraceutical supplements. PUFAs in nutrition were re-emphasized in 1975, when Food and Agriculture organization (FAO) and the World Health Organization (WHO) recommended that infant formula should mimic breast milk composition. From 1990 onwards, many health and nutrition organizations have specific recommendations of addition of ARA and DHA in pre-term and term infant formula (Ward and Singh, 2005).

3. Sources of PUFA and its safety aspect:

PUFAs such as LA and ALA are essential fatty acids, need to be supplied through diet. Currently, the majority of consumed PUFAs are derived from fish and plant oils whereas, fish oil remains the primary commercially available dietary source of long-chain omega-3 PUFAs.Oils extracted from marine fish, such as mackerel, herring, salmon, and sardines, are available in the form of soft gel capsules or oily preparations containing 20% to 30% EPA and DHA. On the flip side, bioaccumulation of fat-soluble contaminants in fish. Also, fishy odor, oxidative degradation of fatty acids and acceptance are some of the problems associated with fish oil supplements. Vegetable oil in artificial fish feed alters the natural fish omega 6/omega 3 ratio and raises the question about its nutritional value forhuman consumption. Concerns about depleting sources, over-fishing and pollution of the marine environment indicated an urgent need for alternative, sustainable sources of LCPUFA. For vegetarian population, dietary sources of omega 3s are limited, are low inlipid content, and therefore unable to contribute any significant amounts of ALA to the diet. Flax seed oil is a good agricultural source of ALA, however, presence of antinutritional factors e.g. cyanogenic glycosides and poor storage stability of oil limits its use. PUFA synthesis in plants shows seasonal variation in synthesis and requires long time for accumulation. Cloning of various desaturases and thioesterases genes in the plants like Arabidopsis, soybean, linseed, tobacco were reported, however transgenic plants for production of

EPA or DHA are yet to appear in market wanting to improvement for acceptability of genetically engineered plants. The typical modern diet, which often includes an abundance of seed oils, tends to provide an excess of omega-6 fatty acids. In today's health landscape, omega-3 fatty acids (specifically EPA and DHA) have taken on the status of being conditionally essential fatty acids, as their consumption is increasingly recognized as crucial for optimal health.The important biological role of omega 3 PUFA, limitations on *de novo* synthesis, the depleting sources and changing lifestyle has generated the demands for omega rich nutraceuticals and pharmaceutical. It is predicted that, for the expanding market the production of PUFA from known current sources will become grossly inadequate.PUFA from fungal oils have appeared on the market e.g. Preterm-infant formula in Europe contains *Mortierella* oil rich in arachidonic acid (ARA), gamma linolenic acid (GLA) rich oil from *Mucor circinelloides* was the first SCO to be produced commercially by JE Sturge, UK.Over the past few decades, widespread research has been carried out for microbial production of PUFA, attained through innovative mutations, genetic manipulations, isolating new strains as well as optimizing the media for cultivating more efficient strains. Microbial systems render unsaturated fatty acids to monohydroxy, dihydroxy and trihydroxy fatty acids. Several algae, bacteria, yeast and fungi are described as potential candidates for PUFA production. *Mucor sp, Mortierella sp, Mortierella* alpine1S-4, and *Cunninghamella sp* have demonstrated the ability to synthesize intracellular lipids from carbohydrates and store them in the mycelium as triacylglycerols. Unfortunately, commercialization of PUFA production using bacteria and microalgae have number of limitations. There are some reports on marine bacterial production of EPA and DHA, but their cultivation at low temperature and at high pressure limits its commercial exploitation. Many marine microalgae are rich in EPA, DHA and do qualify as a promising source of ω-3 fatty acids however, varies with growth conditions, season, and developmental stages.

Detailed survey of fatty acids from diverse algae like Eustigmatophyte, diatoms *P.tricornatum* and red alga *C merolae* revealed that fatty acid biosynthesis is similar and share characteristic of fatty acids from higher plants (Hardwood 2019). Arachidonic acid is dominant component of fats accumulated by brown alga whereas red alga accumulates EPA. Ever increasing demand for PUFA the high value lipid has focused attention on exploring microbial lipid as alternative oils, enriched with PUFAs. Microbial kingdom shows great diversity in PUFA content. Various algae, bacteria, fungi,

and yeast are reported as oleaginous and produce variety of saturated and unsaturated fatty acids.

It is very clear that Yeast, fungi, and algae are the primarily producers of omega-3 in the marine food chain. Consequently, the PUFAs found in marine mammals and fish are often traced back to their origin in marine microbes, phytoplankton, and zooplankton.

In the late 20th century, Ratledge created the term 'SCO' and the microorganisms that accumulate more than 20% lipid as 'oleaginous'. Out of hundreds of yeast species studied, only 25 species and fewer than 50 of the 60,000 fungal species are reported to accumulate more than 20% lipid. Paul Lindner (Berlin, Germany) was first to develop a small-scale process to produce fats from *Trichosporon pullulans*

The first marine yeasts were isolated by Bernhard Fischer in 1894, were identified as *Torula sp. and Mycoderma* sp. Amongst marine oleaginous microorganisms, yeasts are more promising in lipid accumulation than bacteria and phototrophic microalga. Yeasts grow independent of the climatic factors, demonstrate high oleaginicity, use various diverse sugars efficiently, low duplication time as compared to filamentous fungi, are promising microbes for large scale fermentation, (Beopoulos *et al.*, 2010; Ageitos *et al*., 2011). Further, yeasts are relatively more amenable for genetic manipulation and tailor made recombinant yeasts can be designed for higher production of desired PUFA. To promote higher lipid accumulation in yeasts metabolic engineering could be another approach. The typical oily yeast genera include *Lipomyces, Cryptococcus, Yarrowia, Rhodotorula* and *Candida (*Rateledge 2004) and can accumulate 20-80% oil of dry weight.

Unfortunately, oleaginous yeasts typically demonstrate slow growth rate rendering PUFA production a rate-limiting process. The intracellular lipid accumulation in resting cells continues over several days and maximum level is attained however, catabolic pathway remobilizes carbon in fatty acid through β-oxidation. The on -off and duration of each phase depends on the C/N ratio, Accumulation in *Rodotorula glutinis,* oleaginous yeast, in batch mode culture with optimized C/N ratio could result in a 3-fold increased lipid production. Strain improvement is a continuous process and an important prerequisite for improved microbial product realization. For many years, strain improvement was achieved through induced mutations, which was essentially a hit-and-miss approach and essentially random in nature. Moreover, most of the mutagenic agents are toxic, carcinogenic and have deleterious effects. Several genetic mutations affect adversely and therefore need rigorous screening. On the

contrary, metabolic engineering through modern molecular approaches is the practice of altering genes and metabolic pathways with an objective to increase production of a specific substance, which is typically a product of complex metabolic pathway. Oleaginous yeasts (OY) metabolize simple carbon sources, sugars, glycerol, *etc*. andaccumulate neutral lipids, sterols, TAG and Polyunsaturated fatty acids. Under limiting concentration of nitrogen/ phosphorus / magnesium growth of yeasts cell is stopped and starts accumulating lipids. Under nutriment limiting conditions lipid accumulation in OYs as follows:

Stage 1. Production of Acetyl CoA and NADPH

Stage 2. Biosynthesis of Fatty Acid Chains

Stage 3. Allocation of Acyl moieties to either Polar or Neutral Lipid Pools /elongation and unsaturation

Stage 4. Lipid Droplet Biogenesis.

In 1990s, metabolic engineering came into focus for over expression of a particular product. The systematic study of the physiology of particular organism allowed identification of specific metabolic targets and subsequently led to significant improvements in quality and yield of the product. Currently, metabolic engineering is increasingly practiced as a more convenient and cost-effective approach for production of a desired metabolite. Since fish-oil is presently the primary commercially available dietary source of omega-3 fatty acids, containing approximately 20-30% of these beneficial fats. Nonetheless, there are numerous challenges and concerns associated with fish oil supplements. As a result, the exploration for alternative and abundant sources of omega-3 fatty acids has become imperative. Higher plants do not synthesize LCPUFA like DHA and EPA. EPA production reported in transgenic plants like Arabidopsis leaves (3%) and Brassica seeds (25%) (Chen G et al., 2010) but commercial production of EPA from transgenic plants has not yet been achieved. Current research like mutation study, genetic engineering targeting to fulfill the requirement of dietary PUFA. Genetic engineering is one of the promising alternatives to conventional PUFA sources and a powerful and effective approach for enhancing PUFA production. The microbial sources of LCPUFA are long awaited being vegan, free from contamination and without seasonal variations (Bajpai and Bajpai, 1993). Ratledge and Wynn, (2002) have demonstrated that excess carbon is assimilated by cells and is converted into storage fat in nitrogen limiting conditions. However, microorganisms are likely to be important competitors to plants as microorganisms have the advantage of being capable of producing individual PUFAs as the dominant

PUFA in the lipid material. Single cell oil (SCO) containing nutritionally important PUFA from oleaginous micro-organisms is gaining importance occupying its place in market due to the growing awareness of health benefits of PUFAs (Ward and Singh, 2005). In addition, oleaginous yeasts convert numerous raw materials into value added fats and oils.

4. The production of LCPUFA by fermentation:

The production of LCPUFA by submerged fermentation (SmF) using various synthetic media has been explored. According to the expected product, such as oil or biomass for feed, SmF or SSF fermentation is carried out. Media components, mainly the ratio of carbon and nitrogen play an important role during fermentation. To make the process cost effective, components of synthetic media need to be replacedwith economically feasible, easily available agricultural by-products. Considering that agro-industrial by-products are available in abundance in India, SSF has much wider scope over SmF. SSF, defined as the fermentation process in which microorganisms are grown on solid substrate without the presence of free liquid, has drawn interests in industry because of its low wastewater production and operation expenses, superior productivity, and the use of agro-industrial solid residues as substrates Bacteria, yeasts and fungi can grow on solid substrates and find applications in SSF processes. Since SSF occurs when microorganisms grow on solid materials without the presence of free water (Lonsane et al., 1985), only limited microbes can effectively be grown in SSF. The influx of biotechnology, specifically fungal biotechnology, with its inexpensive mode of application, has been used as a tool for the effective conversion of agricultural wastes into useful products. Filamentous fungi can growat low water activity, high osmotic pressure and hyphae can be seen growinginto solid material for better growth and bioconversion of substrates. The fungal fermentation is also gaining attention in industry due to the lowerwastewater production and minimal operating expenses, simple media requirement, superior productivity, and easier prevention of bacterial contamination compared to the submerged fermentation (SmF), in which the nutrients and microorganisms are present in a large amount of water. There are various reports on LCPUFA production by bacteria, yeast, and fungi among which fungal LCPUFA production is widely employed in animal feed due to their growth on low cost or freely available agriculture waste material and higher yield. Under appropriate conditions, filamentous fungi can transform variety of cheaper substrates into high value products (Certik and Adamechova, 2009). Biodegradation of the agro industrialwaste and or by-products can be mediated through microbial processes. Currently solid-state fermented products are more popular as animal

feed. Microbial cultures and fermented products, utilized for fishery and poultry, could enhance omega-3 fatty acid availability in thehuman food chain. In the present study, we have screened oleaginous fungi for production of long chain polyunsaturated fatty acids (LCPUFA). A simple screening method for bioconversion of flax seed meal (FSM) supplied short chain PUFA into LCPUFA using mucor as well as marine yeasts were investigatedwith promising outcome (Jape et al 2016). The value of flax and different agricultural residue could be considerably increased if they are utilized to produce value-added products through fermentation.

Table 1. Conventional and alternative microbial sources for PUFA production

PUFA	Conventional Sources	Microbial Sources
GLA :γ-linolenic acid	Plants (Evening primrose, Borage, Black current)	Fungi- *Mucor circinelloides, Mucor mucedo, Mortierella isabellina, Mortierella ramannina, Cunninghamella echinulata, Cunninghamella elegans, Cunninghamella japonica, Rhizopus arrhizus, Thamnidium elegans* Algae – *Spirulina platensis, Chlorela vulharis*
DGLA :D γ-linolenic acid	Human milk, animal tissues, fish (Scombers crombrus)	mosses *Pogonatum urnigerum* Fungi – *Mortierella spp., especially Mortierella alpina, Conidiobolusna nodes, Saprolegnia ferax*
ARA: arachidonic acid	Animal tissues, fish (Brevoortia, Clupea	mosses *Ctenidium molluscum,* Fungi - *Mortierella spp., especially Mortierella alpina, Conidiobolusna nodes, Entomophthora exitalis, Blastocladiella emersonit* Algae – *Porphyridium cruentum, Sargassums alicifolium, Euglena gracilis*
EPA eicosapentaenoic acid	Fish herring menhaden Shellfish – blue	Fungi – *Mortierella alpina, Mortierella elongata, Pythium irregulare, Pythium ultimum*

	crab, oyster, lobster, mussel	Algae – *Chlorella minutissima, Chlorella minitissima, Monodus subterraneus, Polysiphonia latifolium, Porhyridium cruentum, Phaeodactylum tricornutum, Nannochloropsis oculata, Amphidinium carteri, Thalassiosira pseudonana* Bacteria – *Shewanella putrefaciens*
DHA. docosahexaenoic acid	Fish–tuna, cod, herring, sardine, salmon, crab, mussel, lobster, oyster menhaden Shellfish – blue	*Thraustochytrium aureum, Thraustochytrium roseum, Schyzochytrium SR21, Schyzochytrium aggregatum Microalgae MK 8805, Crypthcodinium cohnii, Gyrodinium nelsoni, Amphidium carteri, Gonyaulax polyedra* Bacteria – *Vibrio spp., Rhodopseudomonas spp*

5. Raw materials for PUFA production using yeasts:

Production of SCO and PUFA using marine microbes has become the focus of intensive research. The commercial viability of this process can be achieved either by increasing the overall lipid yield or by producing lipids that contain high-value fatty acids. In addition to oleagenic nature, various factors like genetic properties of microorganisms, cultivation conditions and media composition decide the success of a fermentation process. Needless to mention, the downstream processing required to materialize the commercially viable yield also needs optimization.

Glucose, Xylose, Lactose as pure form of sugars have been used as sole carbon source for lipid production using oleaginous yeasts. Several studies have reported that various low-cost raw materials as carbon source like cassava, soybean, sugar beet, zero value or negative value waste substrates as carbon source crop residues like wheat bran, rice bran, corn meal, apple pomace could be used to replace glucose in the medium. Tapioca starch, sugar beet molasses orange rind, tomato peels as best source of lipid production in *Cryptococcus curvatus* NRRLY1511 and produced 125g/L biomass that yielded 1.2g/l of oil containing 41.96% saturated fats and 32.13% of PUFA.

Industrial effluents offer a low-costsubstrate for fat production using yeasts. Palm oil mill effluent was tested as potential raw material for production of fats and pigments using oleaginous red yeast *R. glutinis.* Cheese whey by *C. pseudotropicalis*, *A. curvuratum*, oleaginous Zygomycetes. was utilized as renewable substrate for lipid production in various studies *C. bombicola* ATCC 22214 was cultivated on glucose supplemented with soy molasses. *C. albidus*varaerius IBPhMy-229 on medium with ethanol as carbon source produced 63.4 g/l of oil (Evans *et al* 1983).

Y. lipolytica to accumulate lipids was cultivated on diverse substrates like 1% Methanol, 8% molasses, glycerol, Papanikolaou *et al*. 2003) detoxified sugarcane bagasse hydrolysate (DSCBH).

Whey is a potential raw material which can be used as substrate for Large-Scale Production of yeast and considered as a good substrate for production of PUFA and pigments that has application as livestock feed. (Certik 2013). Cheese whey as renewable source for microbial lipid production using zygomycetes is reported by Vamakaki (2010). The production of SCP and SCO rich in saturated fatty acids in shake flasks is reported from cheese whey using kefir microorganisms like *K. fragilis, C. curvurata* etc. Biosynthetic pathway for PUFA synthesis in microbes is well studied, under simple metabolic regulation and easily amenable to modulation through fermentation media and conditions. Further, these oil accumulating biosystems offer wider possibility of modulation through genetic engineering.

Biosynthesis of LCPUFA encompasses a long biochemical pathway initiating at acetyl Co-A as primary precursor and 30 enzymatic reactions. Acetyl moieties are added to growing chain to length of 16 or 18 carbons and further PUFAs are synthesized by modification of saturated fatty acid precursors. During PUFA production, Acetyl-CoA carboxylase and fatty acid synthase, desaturase enzymes catalyse the synthesis and elongation and unsaturation respectively.For example, Δ9 desaturase catalyzes conversion of stearic acid (C18, saturated fatty acid) into oleic acid (monounsaturated fatty acid). Δ12 desaturase converts oleic acid into linoleic acid. Humans cannot synthesize linoleic acid (LA) and linolenic acid (ALA).

Molecular engineering is a promising approach to synthesize economically valuable fatty acids including engineering. These types of studies have mainly focused on maximizing PUFA yield by novel mutations, isolating new strains as well as optimizing the media for cultivating more efficient strains. Genetic engineering of oleaginous strains is another alternative to improve their capacity of lipid storage or to generate lipids with rare fatty acid

profiles. Heterologous expression of Δ6 desaturase gene from Mucorrouxii in Saccharomyces cerevisiae and could produce DHA, gamma-linolenic acid and sterideconic acid.

At present Martek Biosciences Corp. uses *C. cohnii* to manufacture oils that contain high DHA levels for use in infant formulas. Capsules containing DHA are sold as neutraceuticals (Kyle, 1996, http://www.marketbio.com). Xue et al., (2013) communicated first reporton commercial production of EPA from recombinant yeast *Yarrowia lipolytica* Y4305. Recombinant *Lipomyces starkeyi* work has pavedthe way for further development of tailored strains with varying LCPUFA compositions and for developing a resourceful platform to produce other high-value lipid products (Salunke et al 2015). The fatty acid profile of *L. starkeyi* exhibited high content of linoleic acid (LA), perhaps could be a rate limiting situation for LC-PUFA synthesis. Key enzyme Δ15 desaturase catalysis biosynthesis of α-Linolenic acid (ALA) from linoleic acid by facilitating double bond formation at the 3rd carbon from the ω end. Considering the high linoleic acid content, studies (conducted by authors) *L. starkeyi* was chosen as model for metabolic engineering. DHA accumulation in parental strain was considered crucial for selection of strain because DHA (final fatty acid) is stored in triglyceride as an energy source. The systematic study of the physiology of this organism allowed identification of specific metabolic targets and subsequently led to significant improvements in product yield. In non-transformed yeast, this conversion of LA into ALA appears to be rate-limiting resulting in poor accumulation of ALA. The presence of Mucor Δ15 desaturase, alters the metabolic milieu of the yeast by making ALA available as precursor in excess amount. Further, ALA thus produced is utilized and resulted in accumulation of EPA (74.28±0.29mg/l) and DHA (1080±0.37mg/l). Careful selection of host, expression of Δ15 desaturase genes promote host metabolism to improve fatty acid accumulation. Considering environmental safety concerns related to the release of genetically modified organisms, it is essential to develop potential sources of PUFA using natural isolates.

The extensive research and development of PUFA production carried out over the past several years is continuing and is basically aimed at improving the economic competitiveness of microbial lipids compared to plant- and animal-derived oils. The marine oleaginous yeasts are not reported yet for the simultaneous production of both PUFAs and proteins. The whey based solid substrate like whey+ corn, whey + wheat bran, *etc* could provide a promising fermentation system to produce nutritionally enriched feeds. The

whey based solid substrate (whey+ corn, whey+ wheat bran) appears promising for preparing nutritionally enriched products.

In 1980s since cocoa butter was in short supply, preparation of cocoa butter substitute was explored by mutating Δ9 desaturase gene in *Cryptococcus curvatus*. This mutation in Δ9 desaturase gene disallowed conversion of stearate to oleate and caused increased amount of stearate at the expense of oleate that made this oil quite like cocoa butter (Ratledge, 1997). Directed efforts towards Production of higher value polyunsaturated fatty acids (PUFA) through genetic engineering were focused on selecting the non-pathogenic and non-toxin-forming and in GRAS category organisms that do not lose the desirable oil-producing characteristics over time or assume undesirable process or product related characteristics, such that the fermentation process remains consistent from batch to batch.Toxicological studies related to oleaginous yeasts Y. *lipolytica* for Linoleic acid, *Rhodotorula* for carotenoids, Toprina G (*Y. lipolytica*) and Toprina L (*C. tropicalis*) *Lodderomyces elongisporus, Rhodotorula mucilaginosa*, were extensively tested for safety. Generally, the (28-day repeat-dose oral and genetic toxicity study) toxicity studies are conducted on purified potential biomolecule. Safety studies conducted on PUFA products produced with genetically modified (GM) strains of *Y. lipolytica* (Xue*et al.,* 2011) showed no adverse effects. In 2010, DuPont submitted a GRAS Notice for its EPA-enriched oil derived from *Y. lipolytica*to the FDA (http://www.accessdata.fda.gov/ scripts/fcn/gras_notices/ GRN000355.pdf), and sells the product as a dietary supplement under the trade name New Harvest_ (http://www.newharvest.com)

6. PUFA enriched feeds:

Slovak team has done the pioneering work to develop a SSF process where microorganisms belonging to Zygomycetes were used to enrich cereals with PUFA. The fungi cultivated in SSF process cause partial hydrolysis of biopolymers in the substrate. Interestingly, the enzymatic processes decreased the anti-nutrient compounds in cereal substrates to make the ferment more palatable (˘Certik *et al.,* 2010). Mucorales fungi were used (Certik *et al.,* 2013) as potential microbial candidates for enrichment of cereals for simultaneous production of both PUFAs and pigments. Single cell heterotrophs are explored as feed for poultry and marine aquaculture and extensively reviewed by Emerenciano *et al* (2013). Olvera-Novoa (2002) demonstrated *Candida utilis* as a protein source in diets for tilapia. Use of phytoplankton and zooplankton, as starter feed is widely accepted as ‘living capsules of nutrition’ and it is predictable that marine yeasts and algae can be the novel sources of LC-PUFA,

will be soon available at commercial level at a cost that enables wide use as animal and aquaculture feed to restore and bring back LCPUFA in the food chain.

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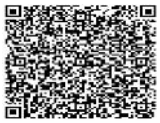	Subject: Biotechnology
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Applied Biotechnology

D. Jansirani * V. Ganapathi Gayathri and K.Muthulakshmi
*Associate Professor, Department of Biotechnology,
P.S.R Engineering College, Sivakasi-626140, Tamil Nadu
E- mail: *jansirani@psr.edu.in*

Introduction

Applied Biotechnology is a field of science that has become increasingly important in recent years with numerous applications in a wide range of industries. It involves the use of biological systems and processes with the aim of developing products and technologies that benefits society. Biotechnology research has opened up new possibilities for addressing pressing global challenges such as climate change, energy insecurity and the need for sustainable food and health care systems.

One of the most significant application of biotechnology is in the **agricultural sector** (Varun Srivastava and Nidhi Srivastava, 2014). Advances in genetic engineering have enabled the development of **genetically modified crops** that are resistant to pests and diseases and can tolerate adverse weather conditions. Biotechnology has also enabled the development of plant that produce higher yields, which can help to address the global food shortage. In the medical field, biotechnology research has contributed to the development of new drugs and therapies, including targeted cancer treatments, vaccines, and gene therapies (Carol A. Miles, 2023).

Biotechnology is also being applied to the field of **energy production**. Biofuels, which are produced from renewable sources such as corn, sugarcane, and algae, offer a sustainable alternative to fossil fuels. They have the potential to reduce greenhouse gas emissions and improve energy security while also creating new economic opportunities. Another key application of biotechnology is in **environmental remediation**. Bioremediation, which involves the use of micro organisms to break down or remove environmental pollution, has shown promising results in cleaning up contaminated sites.

Biotechnology research continues to push the boundaries of science and technology with new developments and applications emerging all the time. The field holds enormous potential for addressing some of the most significant challenges facing humanity, from ensuring food security to combating climate

change. In this long Introduction, we will explore the applications of biotechnology in different industries and sectors and highlight some of the key achievement and challenges in the field (Martin H.G *et al.,* 2005).

Applied biotechnology has emerged as a versatile tool for addressing various challenges in our society. Growing population, climate change, and the ever-increasing need for energy and medical solutions challenge the conventional ways of production and consumptions. It has the potential to provide sustainable and cost-effective solutions to these problems. Biotechnology based products such as biofuels, bioplastics, and biopharmaceuticals offer an alternative that is renewable, biodegradable, eco-friendly. Additionally, it has enabled the development of genetically modified organsims that have revolutionized agriculture, including enhancing crop yields and resistance to pests and diseases. Bioremediation, the use of living organisms to clear the pollutants. Today biotechnology is applied in various ways such as food supply, healthcare, and energy contributing to sustainable development and improving living standards. This chapter reviews some of the key applications of biotechnology and their potential to address some of the most significant challenges facing mankind (Helen Kreuzer and Adrianne Massey., 2005).

One of the main goals of applied biotechnology is to create more efficient and sustainable processes for producing goods and services, while also minimizing negative impacts on environment. For example, by developing new enzymes, micro organisms, and metabolic pathways, researchers have created alternative methods to produce biofuels, textiles, and materials from renewable resources. In **healthcare,** biotechnology has contributed to the development of advanced therapies and treatments for a wide range of diseases and disorders, including cancer, genetic disorder, and chronic illnesses. It has also led to significant breakthroughs in **personalized medicine,** where treatments are tailored based on patient's unique genetic makeup.

Biotechnology has also revolutionized in the field of **biomining and bioleaching** contributing to more efficient and sustainable practices in mining and over processing. These processes utilize the capabilities of living organisms, such as bacteria, fungi, and algae to extract valuable metals from ores or waste materials.

Environmentalists have been looking to biotechnology for controlling the environmental pollution and conservation of nature and natural resources. Efficiency of organisms has been improved with genetic engineering techniques and used to solve environmental problems such as treatment of

waste water coming from industries to avoid water pollution, treatment of oil spills, production of fuels from wastes etc.

Various applications of Biotechnology

1. Biomining and bioleaching:

Biomining is a process that involves the use of microorganisms to extract minerals from ores. This approach is particularly useful for low-grade ores or complex minerals structures that are difficult to process using traditional methods. Microorganisms such as bacteria, are capable of breaking down the ores and releasing the target metals in a soluble form, which can be later recovered and purified.

Bioleaching is a subset of biomining and focuses specifically on the extraction of metals from sulfide ores. Sulfide ores are a common source of valuable metals, but they can also release harmful pollutants when exposed to air and water. Bioleaching utilizes bacteria that naturally occur in the environment or can be specifically introduced to catalyze the oxidation of sulfide minerals, resulting in the dissolution of metals from the ores. It is employed for the recovery of metals such as copper, zinc, lead, arsenic, uranium, cobalt, gold, nickel, molybdenum from ores. And Microbes which are used to leach metals from ores are called bioleachers.

Biotechnology plays a crucial role in biomining and bioleaching by providing methods to enhance the efficiency and effectiveness of these processes. For example, genetic engineering techniques can be used to modify microorganisms and optimize their metabolic pathways to improve metal extraction rates. Researchers are also developing new microbial strains that are more resistant to harsh conditions, such as high temperature or acidic environments, which are often encountered in biomining and bioleaching operations.

Bioleaching was first practiced in the 18th century in **Rio Tinto Mines** to extract copper from ores. Now it is employed to recover metals from low-grade ores, worked out mines, mine dumps and solid waste refuses (tailings). It can be used both inside and outside the mine. The following microbes are used in the bioleaching and biomining field. (Ananthanaryarn and JayaramPaniker., 2009). *Thiobacillus ferrooxidans* (For Cu, Ur, Co, Ni, Zn, Pb), *Thiobacillus acidophilus* (For Fe, Cu), *Thiobacillus organoparpus* (For Fe, Cu), *Thiobacillus thiooxidans (*For Zn, Pb), *Leptospirillum ferrooxidans* (For Fe, Cu), *Sulfolobus acidocaldarius* (For Fe, Cu, Mo) etc.Don Kelly and Warwick proved that combined metal leaching effect of *L. ferrooxidans* and

T.organoparpus is greater than the metal leaching by any of the pure culture. They proved it while leaching copper from the ore chalcopyrite. Fungi such as *Aspergillus niger* and *Penicillium simplicissimus* also solubilize Cu, Pb, Ni, Zn, and Sn. As research and technological advancements continue the potential for biotechnology in these fields is expected to expand even further.

2. Bioremediation:

Biotechnology has revolutionized the field of bioremediation, offering innovative and sustainable solution to address the challenges associated with pollutants in ground level especially in the contaminated areas. Bioremediation is the cleaning up of toxic contaminants in the environment using the activity of microbes. It removes the contaminants from the soil and water. It is a waste management technique. It is mainly concerned with the treatment of hazardous substances such as oil sludges in the soil and radioactive wastes in effluents. Microbes act as Catalysts to reduce the level of hazardness of toxic chemicals. The reduced level of toxic chemicals in the soil and water does not affect the living beings.

Phytoremediation:

A transgenic *Arabidopsis thaliana* with **mercury reductase gene and mercurial lyase gene of bacteria**, was developed in early 2000. This plant **cleans up gold ores and wastesfrom the soil**. Aluminium and arsenic compounds in the soil can be removed by growing transgenic tobacco, papaya, rice, and corn containing Citrate synthetase gene. Citrate synthetase synthesizes citrates in the soil, which increases the solubility of aluminium for easy uptake by the plants. https://www.mdpi.com/1660-4601/14/12/1504

Transgenic tobacco containing nitroreductase gene of *Enterobacter cloacae* degrades the explosive waste TNT in the soil. This plant can tolerate approx. 0.5 mM of TNT in contaminated soils.

Microbial Degradation of Xenobiotic:

Xenobiotics are man-made compounds. They may be biodegradable or persistant. Most agricultural chemicals are xenobiotics. They are usually non-biodegradable. Derivates of heavy metals and biocides are examples of xenobiotics. The toxic chemicals containing heavy metals metals such as mercury, arsenic, cadminum, cobalt, copper, lead, nickel are constituents of effluents from many industries. Biocides, such as 2,4, -D,Benzonate, Parathion, etc. are non-biodegradable and persistant.They are used in agriculture. All these substances cause environmental pollution.

Some specific strains of bacteria can tolerate heavy metals or biocides and degrade them to lose their toxicity. These bacteria are called biodegrading agents. They selectively degrade the toxic substances while almost all other microbes cannot. Mercury compounds are degraded by *Staphylococcus aureus.* https://www.sciencedirect.com/science/article/pii/S0304389419309781.

Corynebacterium flaccumfaciens DGIOI has a plasmid pDGIOI that provide resistance against arsenic compounds. It takes in arsenic compounds along with phosphates and excretes it into the surrounding in a harmless form. Mixed culture of *Alcaligens,Arthrobacter*, *Beijerienckia* and *Pseudomonas pudita* is sprayes on the areas contaminated with Polychlorinated biphenyl compounds (PCBC). PCBC are highly toxic to living beings as their toxicity lasts for a long time.

Bioremediation Methods to Treat Oil Spills:

Some genetically engineered micro-organisms are used to clean up the environment. They include **Superbug(*Pseudomonas putida*).**

Superbug:

Superbug is a **constructed** bacterium. It is a strain of *Pseudomonas putida* that can degrade hydrocarbons founds in petroleum wastes. It ia a multiplasmid strain developed by using genetic engineering techniques. It was developed by **Anand Chakrabarty*et al.*in 1979**. It is used **to treat oil spills** as a measure to control oil pollution. Petroleum products contain Cycloalkenes (octane), naphthenes, xylene, toluene and aromatic hydrocarbons. Since these compounds are not easily biodegradable, oil wastes become a major problem on the soil and water (M. Erusan Rajarajan).

Contruction of Superbug: Identification of parent strains, Conjugative transfer of plasmids, Selection of Superbug, Mass culture.

Identification of Parent Strains:

Charkrabarty *et al.* had succeeded in isolating different strains of *P.putida* that could degrade camphor, octane, xylene, and naphthenes from polluted soils. They found out that each strain has a special type of plasmid thus four types of plasmids are recognized in the bacterial strains. They are CAM plasmid, OCT plasmid, XYL plasmid, NAH plasmid.

Here the CAM plasmid can degrade camphor compounds, OCT plasmid can degrade octane, hexane, decane, XYL plasmid can degrade xylene and toluene, NAH plasmid can degrade naphthenes.

Conjugative Transfer of Plasmids:

The Strain A containg CAM plasmid is joined with Strain B containing OCT plasmid. During this process homologous recombination, takes places so that a large CAM-OCT plasmid is formed. Then this new strain become E. The Strain C containing XYL plasmid is joined with Strain D containing NAH plasmid then the resulting Strain F contain XYL-NAH plasmid. Joining of this two Strains namely E and F to get a desirable Strain.

Selection of Superbug:

Because of Repeated mating the *Pseudomonas* culture has Strain A, B, C, D, E, F and Strain G (Superbug). From this mixture of strains, Strain G is selected by culturing the strains in the presence of all the four pollutants, namely camphor, octane, etc. The superbug alone can grow in the medium. It is subcultured with fresh medium lacking the pollutants for future use.

Mass Culture of Superbug:

The selected Superbug is mass cultured in a suitable liquid medium in a bioreactor for mass production.

Application of Superbug:

A patent was given to Chakrabarty regarding the construction and use of superbug. The American Government in 1990, allowed to use the superbug to clean up oil spills in the water of Texas state. The mass culture of superbug is sprinkled over paddy straw and the straw is dried in shade. The bacteria inoculated straw can be stored for more than a year until we are in need.

To treat oil spill, the straw is spread over the oil slicks and is left as such for a week or more. During this time, the straw soaks up the oil and the bacteria living on it break the oil into non-polluting materials. As a result, the oil wastes become harmless to other organisms living on soil or water polluted with petroleum oil. (Bimal, Rintu Banerjee *et al*., 2010).

Biofuel:

Biofuel is a type of fuel that is derived from organic matter, such as plants and algae, and used as a substitute for traditional fossil fuels, such as oil and gas. Biotechnology – based approaches to biofuel production typically involve the use of microorganisms, such as bacteria, yeast, and algae, to convert **Biomass into Biofuel.** For example, bacterial fermentation can be used to produce bioethanol from sugar-rich crops like corn and sugarcane, while algae can be used to produce biofuel from carbon dioxide and sunlight in

a process known as phototrophic fermentation (Flickinger and Drew, 2013). Genetic engineering can be employed to improve the efficiency and yield of biofuel production, such as by optimizing the metabolic pathways involved in converting biomass to biofuels.

Biogas:

Biogas is a mixture of different gases produced by the breakdown of organic matter in the absence of oxygen. Biogas can be produced from raw materials such as agricultural wastes, manure, municipal wastes, plant materials, sewage, food waste, etc. Biogas is produced under anaerobic condition, when the organic materials are converted though microbiological reactions into gas and organic fertilizer. Biogas primarily consists of methane (63%) along with CO2 and hydrogen. Methane producing bacteria called Methanogens and one such common bacterium is ***Methanobacterium arbophilicum, Methonobacterium mobile, Methanococcus vannielii, Methanosarcina barkeri*** etc. Biogas devoid of smell and burns with a blue flame without smoke. The Methanogens are also present in anaerobic sludge and rumen of cattle. In rumen, these bacteria help in the breakdown of cellulose. The excreta of cattle dung are commonly called 'Gobar'. Gobar gas is generated by the anaerobic decomposition of cattle dung. It consists of methane, CO2 with some hydrogen, nitrogen, and other gases in trace amounts (Herren and Ray., 2005).

In a biogas plant, anaerobic digestion is carried out in an air tight cylindrical tank known as digester. It is made up of concrete bricks and cement or steel. Bio-waste are collected and slurry of dung is fed into this digester. It has a side opening into which organic materials for digestion are incorporated for microbial activity. Anaerobic digestion is accomplished in three stages: solubilization, acidogenesis and methanogenesis. The outlet is connected to a pipe to supply biogas. The slurry is drained through another outlet and is used as fertilizer. Biogas is used for cooking and lighting. The technology of biogas production was developed in India mainly due to the efforts of Indian Agricultural Research Institute (IARI) and Khadi and Village Industries Commission (KVIC). The Cholan Transport Corporation in Tamil Nadu took the first step in February 1992 to run a bus on Biogas.https://www.britannica.com/technology/biogas

Algal biofuel:

Algal fuel also known as Algal Biofuel, or algal oil is an alternative to liquid fossil fuels, the petroleum products. These use algae as a source of energy rich oils. Also, algal fuels are an alternative to commonly known biofuel sources obtained from corn and sugarcane (Bioethanol). The energy crisis and the world food crisis have initiated interest in algal culture (farming algae) for making biodiesel and other biofuels using land unsuitable for agriculture (Ramawat., 2000). *Botryococcus braunii* is normally used to produce algal biofuel.https://www.mdpi.com/1996-1073/12/10/1920

Biodiesel:

Biodiesel is a clean burning oil manufactured from fat or vegetable oils by transesterification process. It is not of petroleum origin but it can be blended with petroleum based diesel for being used as a fuel. It is a **mono-alkyl ester of vegetable oil.** The technique of biodiesel production was invented by G. Chavanne of the University of Brussels (Germany) in 1937. But the commercial production has been stated in the late 1992s.

Biodiesel is manufactured from vegetable oils and fats. The vegetable oil may be obtained from the following sources like Cashewnut, Olive oil, Brazil Nut, Cotton Seeds, Sunflower, Soybean etc. Vegetable oils are esters of fatty acids and glycerol. The oil is methylated by adding methanol, the methylated fatty acid is mixed with excess of alcohol and small amount of sodium hydroxide as a catalyst in a large air-tight container. The methylated fattyacid is converted into methyl ester of fatty acid (biodiesel) and glycerol by transesterification or alcoholysis. The alcohol splits the glycerol from the methyl fatty acid. Here the Methyl ester of fatty acid is the biodiesel and the glycerol is separated with the alcohol fraction and the remaining methyl fatty acid is stored and used as the biodiesel. https://www.britannica.com/technology/biodiesel

Air New Zealand and **Boeing** have sent test flights by loading **Biodiesel** as fuel. ‘ **Biojet fuel**’ refined from biodiesel is used for this purpose. Sometimes biodiesel as also used as a solvent for varnishes.

Bioethanol:

Bioethanol production by biotechnology process involves the use of microorganisms such as bacteria and yeast, to convert renewable resources like sugarcane, corn, or cellulose biomass into bioethanol. This can be done through physical, chemical, or biological methods. Once the sugars are released, they are then fermented by the chosen microorganisms. In the fermentation stage,

the microorganism metabolizes the sugars and converts them into ethanol through a series of biochemical reactions. The most commonly used microorganism for bioethanol production is yeast, specifically strains of ***Saccharomyces cerevisiae, Escherichia coli, Klebsiella oxytoca and Zymomonas mobilis***, etc. These yeast and bacterial strains have been specially engineered to have high ethanol tolerance and maximum ethanol production efficiency (Roger J. Howe, 2018).

After fermentation, the mixture undergoes a separation process to remove impurities and separate the ethanol from the residual biomass. Distillation is typically used to separate the ethanol from water and other byproducts. Dehydration processes may be employed to further remove any remaining water and increase the concentration of ethanol. The final step involves purification to ensure the obtained bioethanol meets the required standards and can be used as a fuel or blended with gasoline. This purification process generally involves molecular sieves or other adsorbents to remove trace impurities. Overall bioethanol production by biotechnology process offers a sustainable alternative to fossil fuels, as it utilizes renewable resources and reduces greenhouse gas emissions. It also contributes to the development of a bio-based economy and reduces dependence on fossil fuels imports.https://www.britannica.com/technology/cellulosic-ethanol.

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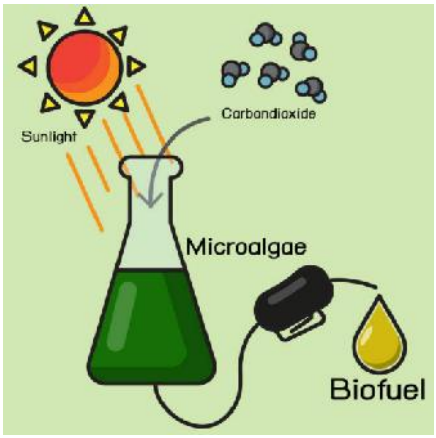

Fig – 01 Algal Biofuel

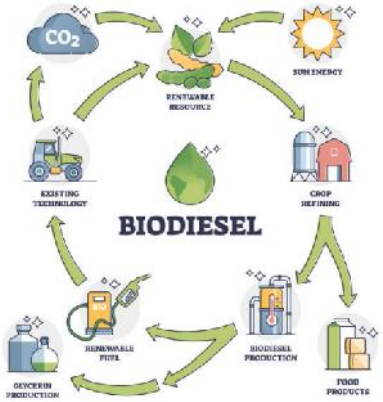

Fig - 02 Biodiesel

Fig – 03 Biogas or Gobar Gas

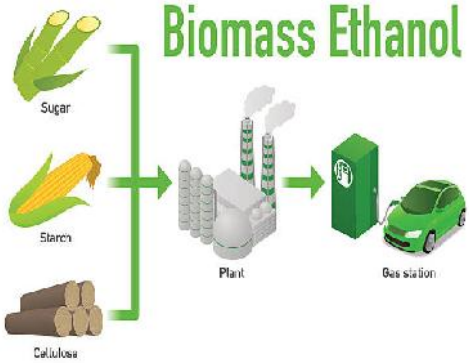

Fig – 04 Bioethanol

Images From https://www.istockphoto.com/vector/microalgae-for-biofuels-gm1401208160-454531929, https://www.istockphoto.com/vector/biofuel-life-cycle-biomass-ethanol-diagram-illustration-gm611750954-105258321?phrase=biogas%20process , https://www.istockphoto.com/vector/biogas-division-diagram-with-digester-generating-gas-fuel-flat-vector-isolated-gm1404900674-456984960?phrase=biogas%20process , https://www.shutterstock.com/search/biodiesel

3. Biotechnology application in Agriculture:

Let us now learn how human beings have used biotechnology to improve the quality of human life especially in the field of food production. The Green Revolution succeeded in tripling the food supply but yet it was not enough to feed the growing population. But in the developing world, farmers are in the critical circumstances to use the agrochemicals although they are too expensive and harmful to the environment but still to improve the yield and so Is there a way to minimize the use of fertilizers and chemicals so that their harmful effects on the environment are reduced? Use of genetically modified crops is a possible solution. One of the application of biotechnology in agriculture that we gonna know in detail are the production of pest resistant plants, which could decrease the amount of pesticide used and somehow may be a determinant for the improvement of yield due to cutting off the pest disturbance (Sanjai Kumar and Sucheta Sinha., 2014).

Bt toxin is produced by a bacterium called ***Bacillus thuringiensis*** (Bt for short). Bt toxin gene has been cloned from the bacteria and been expressed in plants to provide resistance to insects without the needs for insecticides. https://ejbpc.springeropen.com/articles/10.1186/s41938-018-0051-2 In effect created a bio-pesticide. Examples are Bt cotton, Bt corn, Bt brinjal, FlavrSavr Tomato, etc.

Bt Cotton:

Bt Cotton is a genetically modified organisms (GMO) or genetically modified pest resistant plant coton variety which produces an insecticide activity to bollworm. Strains of the bacterium *Bacillus thuringiensis* produce over 200 different Bt toxins, each harmful to different insects. Most Bt toxins are insecticidal to the larvae of moths and butterflies, beetles, cotton bollworms and gatflies but are harmless to other forms of life. The genes are encoded for toxic crystals in the Cry group of endotoxin. When insects attack and eat the cotton plant the Cry toxin are dissolved in the Insect's stomach. https://utcrops.com/cotton/insects-and-mites/biological-control/bt-cotton/

The epithelial membranes of the gut block certain vital nutrients thereby sufficient regulation of potassium ions are lost in the insects and results in the death of epithelial cells in the intestine membrane which leads to the death of the insect larvae.

Bt Brinjal:

The Bt Brinjal is another transgenic brinjal created by inserting a cystal protein gene (Cry1Ac) from the soil bacterium *Bacillus thuringiensis* into the

genome of various brinjal cultivars. The insertion of the gene, along with other genetic elements such as promoters, terminators and an antibiotic resistance marker gene into the brinjal plant is accomplished using *Agrobacterium*-mediated genetic transformation. The Bt Brinjal has been developed to give resistance against *Lepidopteron* insects, in particular the Brinjal Fruit and Shoot Borer (*Leucinodes orbonalis*).https://bteggplant.cornell.edu/bt-eggplant/ (Carl-Erik Tornqvist and William G Hopkins., 2006)

FlavrSavr Tomato:

Agrobacterium mediated genetic engineering technique was followed to produce Flavr-Savr tomato i.e., retaining the natural colour and flavour of tomato. Through genetic engineering the ripening process of the tomato is slowed down and thus prevent it from softening and to increase the shelf life. The tomato was made more resistant to rotting by Agrobacterium mediated gene transfer mechanism of introducing an antisense gene which interferes with the production of the enzyme polygalacturonase, which help in delaying the ripening process of tomato during long storage and transportation.The FLAVR SAVR™ tomato (LYCOPERSICON ESCULENTUM) was developed through a specific genetic modification to exhibit decreased polygalacturonase (PG) activity. https://www.canada.ca/en/health-canada/services/food-nutrition/genetically-modified-foods-other-novel-foods/approved-products/suppressed-polygalacturonase-activity-flavr-savr-tomato.html

Fig – 05 Bt Brinjal

Fig – 06 Bt Cotton

Fig – 07 FlavrSavr Tomato

Images from https://www.istockphoto.com/photo/american-cotton-field-gm492733382-76481059 ,

https://www.istockphoto.com/photo/eggplants-isolated-gm173879887-9404751?phrase=brinjal ,

https://www.istockphoto.com/photo/tomatoes-on-the-vine-gm847335116-138912885?phrase=tomatoes

4. Genetically engineered Insulin:

The Recombinant DNA technological processes have made immense impact in the area of health care by enabling mass production of safe and more effective therapeutic drugs. At present about 30 recombinant therapeutics have been approved for human use the world over. In India around 12 of these are presently being marketed. Management of adult-onset diabetes is possible by taking insulin at regular time intervals. What would a diabetic patient do if enough human-insulin was not available? If we see this in depth, we would soon realize that one would have to isolate and use insulin from other animals (Garg and Anisha Gupta., 2016).

Actually the human Insulin is synthesized by the Beta cells of Islets of Langerhans in the pancreas. It is formed f 51 aminoacids which are arranged in two polypeptide chains, A and B. The polypeptide chain has A has 21 amino acids while the polypeptide chain B has 30 amino acids. Both A and B chains are attached together by disulphide bonds. Insulin controls the levels of glucose in blood. It facilitates the cellular uptake and utilization of glucose for the release of energy. Deficiency of insulin leads to diabetes mellitus which is characterized by increased blood glucose concentration and a complex of symptoms which may lead to death. If untreated. A continuous program of

insulin dependence is required to treat this deficiency (Smita Rastogi and Neelam Pathak., 2010).

In the early years, insulin isolated and purified from the pancreas of slaughtered pigs and cows was used to treat diabetic patients. Due to minor differences in the structure of the animal's insulin as compared to human insulin, it resulted in the occurrence of allergic reactions in some diabetic patients. Production of insulin by recombinant DNA technology started in the late 1970s. This technique involved the insertion of human insulin gene on the plasmid of *E.coli.* The polypeptide chains are synthesized as a precursor called pre-pro insulin, which contains A and B segments linked by a third chain (C) and preceded by a leader sequence. The leader sequence is removed after translation and the C chain is excised leaving the A and B polypeptide chains.

Insulin was the first ever pharmaceutical product of recombinant DNA technology administered to humans. The approval to use recombinant insulin for diabetes mellitus was given in 1982. In 1986 human insulin was marketed under the trade name Humulin. Best and Banting in 1921 isolated insulin from the pancreatic islets of a dog and demonstrated its effectiveness against diabetes (Moriya *et al*.,2008)

Eli Lilly prepared the two DNA sequences complementary to the A chain and B chain of insulin. These DNA sequences were incorporated in the plasmids of *E. coli*. These two chains were synthesizing separately, extracted and combined by disulphide bonds to form genetically engineered human insulin.

The first genetically engineered insulin was developed by Dr. Herbert Boyer and Dr. Stanley Cohen in 1978, and it was approved by the US Food and Drug Administration (FDA) in 1982 after human trials. Since then, genetically engineeredinsulin has become the standard treatment for diabetes worldwide, helping millions of peopleto manage their condition effectively and lead everyday lives.

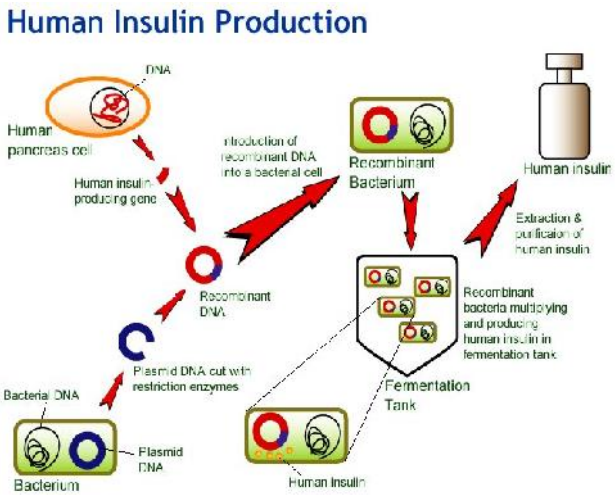

Fig - 8 Image From https://studylib.net/doc/25340981/human-insulin-production

5. Molecular diagnostics:

Early diagnosis of infectious diseases or inherent genetic defects is essential for appropriate treatment. Early detection of the disease is not possible using conventional methods like microscopic examinations, serum analysis and urine analysis. These lab techniques are indirect and not always specific. Scientists are continuously searching for specific sensitive and simple diagnostic techniques for diagnosis of diseases. Recombinant DNA technology, Polymerase Chain Reaction (PCR) and Enzyme linked Immunosorbent Assay (ELISA) are some of the techniques that are reliable and help in early diagnosis.https://www.hindawi.com/journals/bmri/2014/653014/. Presence of pathogens like virus, bacteria, etc. is detected only when the pathogen produces symptoms in the patient. By the time the symptoms appear concentration of pathogen becomes very high in the body. Howeve very low concentration of a bacteria or virus, even when the symptoms of the disease do not appear, can be detected by amplification of their nucleic acid.

ELISA (Enzyme Linked Immunosorbent Assay):

ELISA is a biochemical procedure discovered by **Eva Engvall** and **Peter Perlmanin** (1971) to detect the presence of specific antibodies or antigens in a sample of serum, urine etc. It is a very important diagnostic tool to determine if a person is HIV positive or negative, ELISA is a tool for determining serum antibody concentrations (such as the antibodies produced in a person infected by pathogens such as HIV) and also for detecting the

presence of specific antigens and hormones such as human chorionic gonadotropins (David. R Heldman, 2004).

During diagnosis the sample suspected to contain the antigen is immobilized on the surface of an ELISA plate. The antibody specific to this antigen is added and allowed to react with the immobilized antigen. The anti-antibody is linked to an appropriate enzyme like peroxidase. The unreacted antibody is washed away and the substrate of the enzyme (hydrogen peroxidase) is added with certain reagents such as 4-chloronaphthol. The activity of the enzyme yields a coloured product indicating the presence of the antigen. The intensity of the colour is directly proportional to the amount of the antigen. ELISA is highly sensitive and can detect antigens in the range of a nanogram(Atlas , Richard Bartha *et al*.,2012)

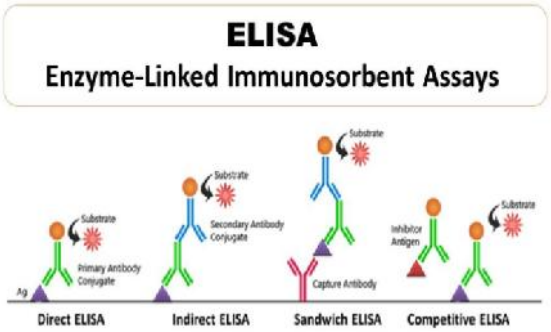

Fig – 9 Images From https://www.biomol.com/resources/applications/elisa/#

PCR (Polymerase Chain Reaction):

The polymerase chain reaction (PCR) is an *invitro* amplification technique used for synthesizing multiple identical copies (billions) of DNA of interest. The technique was developed by **Kary Mullis** (Nobel laureate, 1993) in the year 1983.

Denaturation, renaturation or primer annealing and synthesis or primer extension, are the three steps involved in PCR. The double stranded DNA of interest is denatured to separate into two individual strands by high temperature. This is called denaturation. Each strand is allowed to hybridize with a primer template is used to synthesize DNA by using Taq-DNA polymerase. During denaturation the reaction mixture is heated to 95◦C for a short time to denature the target DNA into single strands that will act as a

template for DNA synthesis. Annealing is done by rapid cooling of the mixture, allowing the primers to bind to the sequences on each of the two strands flanking the target DNA. During primer extension or synthesis, the temperature of the mixture is increased to 75◦C for a sufficient period of time to allow Taq DNA polymerase to extend each primer by copying the single stranded template. At the end of the incubation both single template strands will be made partially double stranded. The new strand of each double stranded DNA extends to a variable distance downstream. These steps are repeated again and again to generate multiple forms of the desired DNA. This process is also called DNA amplification (Glick, B.R. and Pasternak, J.J.,2002).

The PCR technique can also be used for amplifications of RNA in which case it is referred to as reverse transcription PCR (RT-PCR). In this process the RNA molecules (mRNA) must be converted to complementary DNA by the enzyme reverse transcriptase. The cDNA then serves as the template for PCR.https://academic.oup.com/clinchem/article-abstract/43/11/2021/5640827

Applications of PCR:

The differences in the genomes of two different organisms can be studied by PCR. PCR is very important in the study of evolutions, more specifically phylogenetics. As a technique which can amplify even minute quantities of DNA from any source, like hair, mummified tissues, bones or any fossilized materials. (Bernard R. Glick *et al*., 2010).

PCR technique can also be used in the field of forensic medicine. A single molecule of DNA from blood strains, hair, semen of an individual is adequate for amplification by PCR. The amplified DNA is used to develop DNA fingerprint which is used as an important tool in forensic science. Thus PCR is very useful for identification of criminals. PCR is also used in amplification of specific DNA segment to be used in gene therapy.

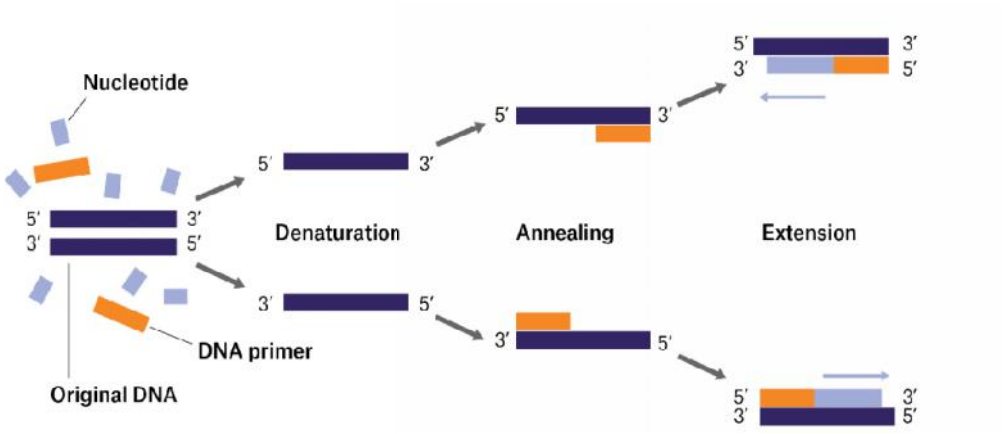

Fig – 10 (Images From https://www.mix.com/add?url=https://blog.labtag.com/a-brief-history-of-pcr-and-its-derivatives/

6. Gene therapy:

If a person is born with a hereditary disease, can a corrective therapy be taken for a such a disease? Yes, Advancement in field of Biotechnology offers an application namely Gene therapy. Gene therapy is an attempt to do this. Gene therapy is a collection of methods that allows correction of a gene defect that has been diagnosed in a child/embryo. Here genes are inserted into a person's cells and tissues to treat a disease. Correction of a genetic defect involves delivery of a normal gene into the individual or embryo to take over the function of and compensate for the non-functional gene. currently, more than 1800 approved gene therapy clinical trials worldwide have been conducted or are still ongoing (Clary., 2010).

The first clinical gene therapy was given in 1990 by French Anderson to a 4-year-old girl with adenosine deaminase (ADA) deficiency. ADA deficiency or SCID (Severe combined immunodeficiency) is an autosomal recessive metabolic disorder. It is caused by the deletion or dysfunction of the gene coding for ADA enzyme. In these patients the nonfunctioning T-lymphocytes cannot elicit immune responses against invading pathogens. The right approach for SCID treatment would be to give the patient a functioning ADA which breaks down toxic biological products.https://www.sciencedirect.com/science/article/pii/S0378111913004344

In some children ADA deficiency could be cured by bone marrow transplantation, where defective immune cells could be replaced with healthy immune cells from an donor. In some patients it can be treated by enzyme replacement therapy, in which functional ADA is injected into the patient. (Madhavan *et al*., 2009).

During gene therapy the lymphocytes from the blood of the patient are removed and grown in a nutrient culture medium. A healthy and functional human gene, ADA cDNA encoding this enzyme is introduced into the lymphocytes using a retrovirus. The genetically engineered lymphocytes are subsequently returned to the patient. Since these cells are not immortal, the patient requires periodic infusion of such genetically engineered lymphocytes. The disease could be cured permanently if the gene for ADA isolated from bone marrow cells are introduced into the cells of the early embryonic stages.

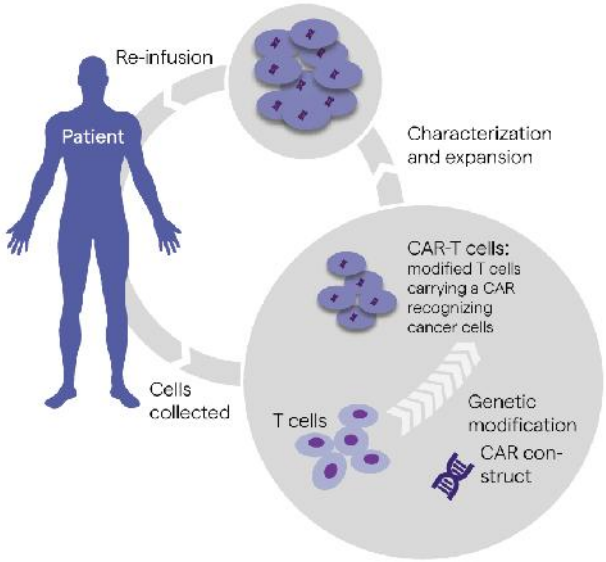

Fig – 11 Image From https://bioscience.lonza.com/lonza_bs/CH/en/cell-and-gene-therapy

Conclusion

In conclusion, applied biotechnology has emerged as a powerful tool that has revolutionized various sectors, including healthcare, agriculture, and the environment. Through the manipulation of biological systems, biotechnology has unlocked numerous possibilities in term of diagnostic techniques, therapeutic advancements, and sustainable solutions.

In the field of healthcare, biotechnology has enabled the development of new vaccines, medicines, and treatments. It has facilitated the production of recombinant proteins, monoclonal antibodies, and gene therapies, which have revolutionized the treatment of various diseases, such as cancer, diabetes and genetic disorders. Biotechnology has also played a significant role in the development of personalized medicine, allowing for more precise and targeted treatments based on an individual's genetic makeup (William J. Thieman and Michael a Palladino., 2004).

In agriculture, Biotechnology has contributed to the enhancement of crop yield, nutritional quality and pest and disease resistance. Genetically modified crops, such as insect-resistant Bt cotton, Bt Brinjal and herbicide tolerant soyabeans, have significantly improved agricultural productivity and reduced the need for chemical pesticides. Biotechnology has also facilitated the development of biofortified crops with improved nutritional profiles helping to address malnutrition and food insecurity in many parts of the world.

Furthermore, Biotechnology has been applied to address environmental challenges and promote sustainability. It has allowed for the development of biofuels, such as ethanol and biodiesel as alternatives to fossil fuels, thereby reducing greenhouse gas emissions and dependence on non-renewable energy sources. Biotechnology has also been employed in bioremediation processes to clean up contaminated environment and mitigate the impact of pollutants (R.C. Dubey, 2003).

However, the application of biotechnology should not be taken lightly. Ethical, social and environmental consideration must be taken into account to ensure its responsible use. The potential risks and unintended consequences associated with genetically modified organisms and gene editing technologies require thorough risk assessments and regulatory frameworks.

Moreover, concerns related to food safety, biodiversity and the concentration of power in the hands of a few multinational corporations need to be addressed. Transparency, public engagement, and informed decision-making are crucial to ensure the responsible and sustainable application of biotechnology.

In a nutshell Biotechnology has undoubtedly brought numerous benefits to society, from improved healthcare to sustainable agriculture and environmental solutions. However, its application needs to be guided by ethical principles, scientific evidences and a consideration of the broader societal and environmental implications. Continuous research, innovation, and public

dialogue are necessary to unlock the full potential of biotechnology while addressing the challenges it presents.

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Cancer: Treatment, Prevention and Management

K. Ajintha[1], Dr. J. Shifa Vanmathi[2*]
[1]Research Scholar, Department of Zoology,
Sadakathullah Appa College (Autonomous), Rahmath Nagar,
Tirunelveli-627011 Affiliated to Manonmaniam Sundaranar University,
Tirunelveli, Tamilnadu, India. E.mail: *ajinthak97@gmail.com*
[2]Assistant professor, Department of Zoology,
Sadakathullah Appa College (Autonomous), Rahmath Nagar,
Tirunelveli-627011 Affiliated to Manonmaniam Sundaranar University,
Tirunelveli, Tamilnadu, India. E.mail: *dijofebi@gmail.com*

Abstract

In the world, cancer is one of the main causes of death. Fortunately, routine screening can identify a number of cancer forms, including colorectal, breast, and prostate cancer. This allows for the early identification of dangerous lesions.A combination of environmental and genetic factors, including behavioral, lifestyle, and environmental exposures, affect cancer risk. Prevention is defined as “the protection of health by personal and community-wide efforts”. These efforts are achieved by describing the burden of cancer, identifying its causes, and evaluating and implementing cancer prevention interventions.

Introduction

Cancer is a disease that occurs when a few of the body's cells grow out of control and spread to other parts of the body. In the millions of cells that make up the human body, cancer can develop almost anywhere.

The second leading cause of death worldwide is cancer. But compared to 20 years ago, fewer individuals are passing away from cancer. Early detection and innovative treatments are treating cancer and extending the lives of cancer patients. In order to help patients avoid getting cancer, medical researchers are simultaneously finding separate risk factors associated to the disease.

Difference between a normal cell and a cancerous cell

Normally, cells follow instructions provided by genes. Genes set down rules for cells to follow, such as when to start and stop growing. Cancerous cells ignore the rules that normal cells follow:

- Normal cells divide and multiply in a controlled manner. Cancerous cells multiply uncontrollably.
- Normal cells are programmed to die (apoptosis). Cancerous cells ignore those directions.
- Normal cells for solid organs stay put. All cancerous cells are able to move around.
- Normal cells don't grow as fast as cancerous cells.

How does cancer start in body?

Cancer starts when a gene or several genes mutate and create cancerous cells. These cells create cancer clusters, or tumors. Cancerous cells may break away from tumors, using lymphatic system or bloodstream to travel to other areas of your body. (Healthcare providers call this metastasis.)

For example, a tumor in breast may spread to lungs, making it hard to breathe. In some types of blood cancer, abnormal cells in bone marrow make abnormal blood cells that multiply uncontrollably. Eventually, the abnormal cells crowd out normal blood cells.

The most common cancers are

- **Breast cancer**: Breast cancer can occur in women and rarely in men.symptoms of breast cancer include a lump in the breast, bloody discharge from the nipple and changes in the shape or texture of the nipple or breast.

  Its treatment depends on the stage of cancer. It may consist of chemotherapy, radiation, hormone therapy and surgery.

- **Lung cancer**: Lung cancer is the second most common cancer. Lung cancer, also known as lung carcinoma, is a malignant tumor that begins in the lung. Lung cancer is caused by genetic damage to the DNA of cells in the airways, often caused by cigarette smoking or inhaling damaging There are two types of lung cancer: non-small cell cancer and small cell lung cancer.

- **Prostate cancer**: Prostate cancer is the uncontrolled growth of cells in the prostate, a gland in the male reproductive system just below the bladder. Early prostate cancer usually causes no symptoms. As the tumor grows, it can damage nearby organs causing erectile dysfunction, blood in the urine or semen, and trouble urinating.
- **Colorectal cancer**: *Colorectal cancer* is a disease in which cells in the colon or rectum grow out of control. Sometimes it is called *colon cancer*, for short. The colon is the large intestine or large bowel. The rectum is the passageway that connects the colon to the anus.Sometimes abnormal growths, called *polyps*, form in the colon or rectum. Over time, some polyps may turn into cancer.
- **Blood cancers**: Leukemia and lymphoma are the most common blood cancers. Most blood cancers, also called hematologic cancers, start in the bone marrow, where blood cells are produced. Blood cancers occur when abnormal blood cells grow out of control, interfering with the function of normal blood cells, which fight off infection and produce new blood cells.

Symptoms and Causes

Symptoms of Cancer

Some common early cancer symptoms include:

- Unexplained weight loss.
- Chronic tiredness.
- Persistent pain.
- Fever that occurs mostly at night.
- Skin changes, particularly moles that change shape and size or new moles.

Left untreated, cancer may cause additional symptoms, including:

- Bruising or bleeding more easily.
- Lumps or bumps under your skin that don’t go away.
- Difficulty breathing.
- Difficulty swallowing.

Causes of cancer

Cancer is a genetic disorder. It happens when genes that manage cell activity mutate and create abnormal cells that divide and multiply, eventually disrupting how your body works.

Medical researchers estimate 5% to 12% of all cancers are caused by inherited genetic mutations that can't control.

More frequently, cancer happens as an acquired genetic mutation. Acquired genetic mutations happen over the course of life. Medical researchers have identified several risk factors that increase chance of developing cancer.

Reduce risk of developing cancer

We can reduce our risk by changing some of our lifestyle choices:

- If we smoke or use tobacco, try to stop. Ask a healthcare provider about smoking cessation programs that can help we quit tobacco.
- Follow a diet plan that's healthy for us. If we want help managing our weight, ask a healthcare provider about nutritional guidance and weight management programs.
- Add exercise to your daily routine. Exercise may boost our immune system so it provides more protection against cancer.
- Avoid toxins, including asbestos, radon and pesticides.
- Protect ourself against sun damage.
- Have regular cancer screenings.

Diagnosis and tests

Diagnosis of cancer

Healthcare providers begin a cancer diagnosis by doing a comprehensive physical examination. They'll ask to describe our symptoms. They may ask about our family medical history. They may also do the following tests:

- Blood tests.
- Imaging tests.
- Biopsies.

Blood tests

Blood tests for cancer may include:

- **Complete blood count (CBC)**: A CBC test measures and counts your blood cells.
- **Tumor markers**: Tumor markers are substances that cancer cells release or that your normal cells release in response to cancer cells.
- **Blood protein tests**: Healthcare providers use a process called electrophoresis to measure immunoglobulins. Your immune system reacts to certain cancers by releasing immunoglobulins.
- **Circulating tumor cell tests**: Cancerous tumors may shed cells. Tracking tumor cells helps healthcare providers monitor cancer activity.

Imaging tests

Imaging tests may include:

- **Computed tomography (CT) scan**: CT scans check for cancerous tumors' location and impact on organs and bones.
- **X-rays**: X-rays use safe amounts of radiation to create images of your bones and soft tissues.
- **Positron emission test (PET) scan**: PET scans produce images of organs and tissues at work. Healthcare providers may use this test to detect early signs of cancer.
- **Ultrasound**: An ultrasound uses high-intensity sound waves that show structures inside of body.
- **Magnetic resonance imaging (MRI)**: MRIs use a large magnet, radio waves and a computer to create images of organs and other structures inside of body.
- **Iodine meta-iodobenzylguanidine (MIGB)**: This nuclear imaging test helps detect cancer, including carcinoid tumors and neuroblastoma.

Biopsies

A biopsy is a procedure healthcare providers do to obtain cells, tissue, fluid or growths that they'll examine under a microscope. There are several kinds of biopsies:

- **Needle biopsy**: This test may be called a fine needle aspiration or fine needle biopsy. Healthcare providers use a thin hollow needle and

syringe to extract cells, fluid or tissue from suspicious lumps. Needle biopsies are often done to help diagnose breast cancer, thyroid cancer or cancer in lymph nodes.

- **Skin biopsy**: Healthcare providers remove a small sample of skin to diagnose skin cancer.
- **Bone marrow biopsy**: Healthcare providers remove a small sample of bone marrow so they can test the sample for signs of disease, including cancer in bone marrow.
- **Endoscopic or laparoscopic biopsy**: These biopsies use an endoscope or laparoscope to see the inside of body. With both of these methods, a small cut is made in skin and an instrument is inserted. An endoscope is a thin, flexible tube with a camera on the tip, along with a cutting tool to remove sample. A laparoscope is a slightly different scope.
- **Excisional or incisional biopsy**: For these open biopsies, a surgeon cuts body and either the entire tumor is removed (excisional biopsy) or a part of the tumor is removed (incisional biopsy) to test or treat it.
- **Perioperative biopsy**: This test may be called a frozen section biopsy. This biopsy is done while you're having another procedure. Your tissue will be removed and tested right away. Results will come in soon after the procedure, so if you need treatment, it can start immediately.

Genetic testing

Cancer may happen when a single gene mutates or several genes that work together mutate. Researchers have identified more than 400 genes associated with cancer development. People who inherit these genes from their biological parents may have an increased risk of developing cancer. Healthcare providers may recommend genetic testing for cancer if you have an inherited form of cancer. They may also do genetic testing to do therapy that targets specific cancer genes. They use test results to develop a diagnosis. They'll assign a number or stage to your diagnosis. The higher the number, the more cancer has spread.

Determination of cancer stage

Healthcare providers use cancer staging systems to plan treatment and develop a prognosis or expected outcome. TNM is the most widely used cancer staging system. T stands for primary tumor. N stands for lymph nodes and indicates whether a tumor has spread to your lymph nodes. M stands for metastasis, when cancer spreads.

Stages of cancer

Most cancers have four stages. The specific stage is determined by a few different factors, including the tumor's size and location:

- Stage I: The cancer is localized to a small area and hasn't spread to lymph nodes or other tissues.
- Stage II: The cancer has grown, but it hasn't spread.
- Stage III: The cancer has grown larger and has possibly spread to lymph nodes or other tissues.
- Stage IV: The cancer has spread to other organs or areas of body. This stage is also referred to as metastatic or advanced cancer.

Though stages one through four are the most common, there's also a Stage 0. This earliest phase describes cancer that's still localized to the area in which it started. Cancers that are still in Stage 0 are usually easily treatable and are considered pre-cancerous by most healthcare providers.

Management and treatment

Healthcare providers may use several different treatments, sometimes combining treatments based on your situation. Common cancer treatments include:

- **Chemotherapy**: Chemotherapy is one of the most common cancer treatments. It uses powerful drugs to destroy cancer cells. You may receive chemotherapy in pill form or intravenously (through a needle into a vein). In some cases, providers may be able to direct chemotherapy to the specific area affected.
- **Radiation therapy**: This treatment kills cancer cells with high dosages of radiation. Healthcare provider may combine radiation therapy and chemotherapy.
- **Surgery**: Cancerous tumors that haven't spread may be removed with surgery. Healthcare provider may recommend therapy. This treatment combines surgery with chemotherapy or radiation to shrink a tumor before surgery or to kill cancer cells that may remain after surgery.
- **Hormone therapy**: Sometimes, providers prescribe hormones that block other cancer-causing hormones. For example, men and people assigned male at birth who have prostate cancer might receive

hormones to keep testosterone (which contributes to prostate cancer) lower than usual.

- **Biological response modifier therapy**: This treatment stimulates immune system and helps it perform more effectively. It does this by changing your body's natural processes.
- **Immunotherapy for cancer**: Immunotherapy is a cancer treatment that engages immune system to fight the disease. The treatment may be called biological therapy.
- **Targeted therapy for cancer**: Targeted therapy is a cancer treatment that targets the genetic changes or mutations that turn healthy cells into cancer cells.
- **Bone marrow transplant**: Also called stem cell transplantation, this treatment replaces damaged stem cells with healthy ones. Autologous transplantation uses supply of healthy stem cells. Allogeneic transplantation uses stem cells donated by another person.

Cancer treatment side effects

Healthcare providers work to balance the treatment so it destroys cancer without harmful or lasting side effects. Even so, all cancer treatments have side effects. Some treatments cause side effects that last for years after treatment is completed. Many people benefit from palliative care that eases cancer symptoms and treatment side effects. The most common cancer treatment side effects are:

- Anemia.
- Nausea and vomiting.
- Fatigue.
- Pain.

Prognosis

What is the prognosis for cancer?

Right now, more people are being cured of cancer or living longer with cancer. In general, people with cancer that were diagnosed and treated before it could spread have a good outlook.

Healthcare providers will base prognosis on factors such as:

- Overall health.

- Having the type of cancer .
- The stage of cancer.
- How it may respond to treatment.

Cancer survival rates

Survival rates are estimates based on the experiences of large groups of people who have different kinds of cancer. Like prognoses, cancer survival rates vary based on cancer type, stage and treatment. According to the most recent data from the National Cancer Institute, 68% of people with any kind of cancer were alive five years after their diagnosis.

Living With Cancer?

Self-care is an important part of living with cancer. Some self-care suggestions include:

- Establish good eating and exercise habits. Ask to speak with a nutritionist for healthy menu ideas.
- Fatigue is a common symptom and treatment side effect. Pay attention to your body and rest when you need to, not just when you can.
- People may be living with cancer for a long time. That is good news, of course, but chronic illness may be challenging. Talking to a mental health professional or finding a support group may help navigate challenges.

Cancer survivorship

If people have cancer, they are a cancer survivor. Cancer survivorship starts the day they receive a cancer diagnosis and continues for the rest of theirlife. As a cancer survivor, they are likely to experience many challenges or complications.

Cancer that comes back

Sometimes, cancer treatment doesn't eliminate all cancerous cells. Those cells can become new cancerous tumors. Cancer that comes back, or recurrent cancer, may appear at the same place as the original cancer, in nearby lymph nodes or spread to organs and tissues far away from the original site.

Second cancer

A second cancer is a new cancer. People who have second cancers may have cancer in the same organ or area of their body as the first cancer, but it is

a different type of cancer from what it was before. They may also have cancer in different areas of their bodies. Second cancers are more common, as more people live longer with cancer.

Cancer fatigue

Cancer fatigue is an overwhelming sense of tiredness that isn't helped by getting more rest. Some people have chronic cancer fatigue that continues after they've finished treatment.

Cancer pain

Some cancer treatments have lasting side effects that may cause pain. One study found that 39% of people who completed cancer treatment had chronic pain. Peripheral neuropathy is an example of pain that may persist after treatment.

Chemotherapy brain fog

Chemotherapy brain fog (chemo brain) happens when cancer or cancer treatment affects their ability to remember or act on information. Approximately 75% of cancer patients report having memory, concentration and their ability to complete tasks.

When they need for healthcare provider?

Any problems they have while receiving cancer treatment should be discussed with their healthcare provider

- A fever of 101 degrees Fahrenheit (38.33 degrees Celsius) or higher.
- Severe headaches.
- Chills.
- Persistent cough.
- Shortness of breath (dyspnea).
- Sores on your lips or in your mouth.
- Sudden weight loss greater than five pounds.
- Excessive vomiting (three times an hour for three hours or more).
- Blood in your urine (pee) or feces (poop).
- Excessive bleeding or bruising.

Prevention of Cancer :

Between 30 - 50% of cancer cases can be avoided. The most economical long-term approach to cancer control is prevention. In addition to ensuring that people are given the knowledge and assistance they need to adopt healthy lifestyles, WHO works with Member States to develop national policies and programmes aimed at increasing exposure to and decreasing awareness of cancer risk factors.

Tobacco

Worldwide, tobacco use is the single greatest avoidable risk factor for cancer mortality and kills more than 8 million people each year, from cancer and other diseases.

Alcohol

Alcohol use is a risk factor for many cancer types including cancer of the oral cavity, pharynx, larynx, oesophagus, liver, colorectal and breast. Risk of cancer increases with the amount of alcohol consumed. Globally, 1 in 20 breast cancers is attributed to alcohol consumption.

Physical inactivity, dietary factors, obesity and being overweight

Overweight and obesity are linked to many types of cancer such as oesophagus, colorectal, breast, endometrial and kidney. Regular physical activity, and maintaining a healthy body weight, and a healthy diet can risk.

Infections

Cancer causing infections, such as hepatitis and human papilloma virus (HPV), are responsible for up to 25% of cancer cases in low- and middle-income countries Vaccines are available for hepatitis B virus and some types of HPV, and can reduce the risk of liver and cervical cancers, respectively.

Environmental pollution

Nearly 4 million people die prematurely from illness attributable to the household air pollution from cooking with solid fuels and kerosene.

Hormone therapy

Women taking hormone replacement therapy may have an increased risk for breast cancer and endometrial cancer.

Radiation

Exposure to all types of ionizing radiation increases the risk of various types of malignancy including leukaemia and a number of solid tumours. Risks increase when the exposure occurs at a young age and also when the exposure amount is higher. Ultraviolet (UV) radiation, and in particular solar radiation, is carcinogenic to humans, causing all major types of skin cancer.Avoiding excessive exposure, and using sunscreen and protective clothing are effective preventive measures.

"Cancer is only going to be a chapter in your Life, not the whole story."

—JOE WASSER

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