THE MICROBIAL NEXUS

INTERSECTIONS IN MODERN SCIENCE

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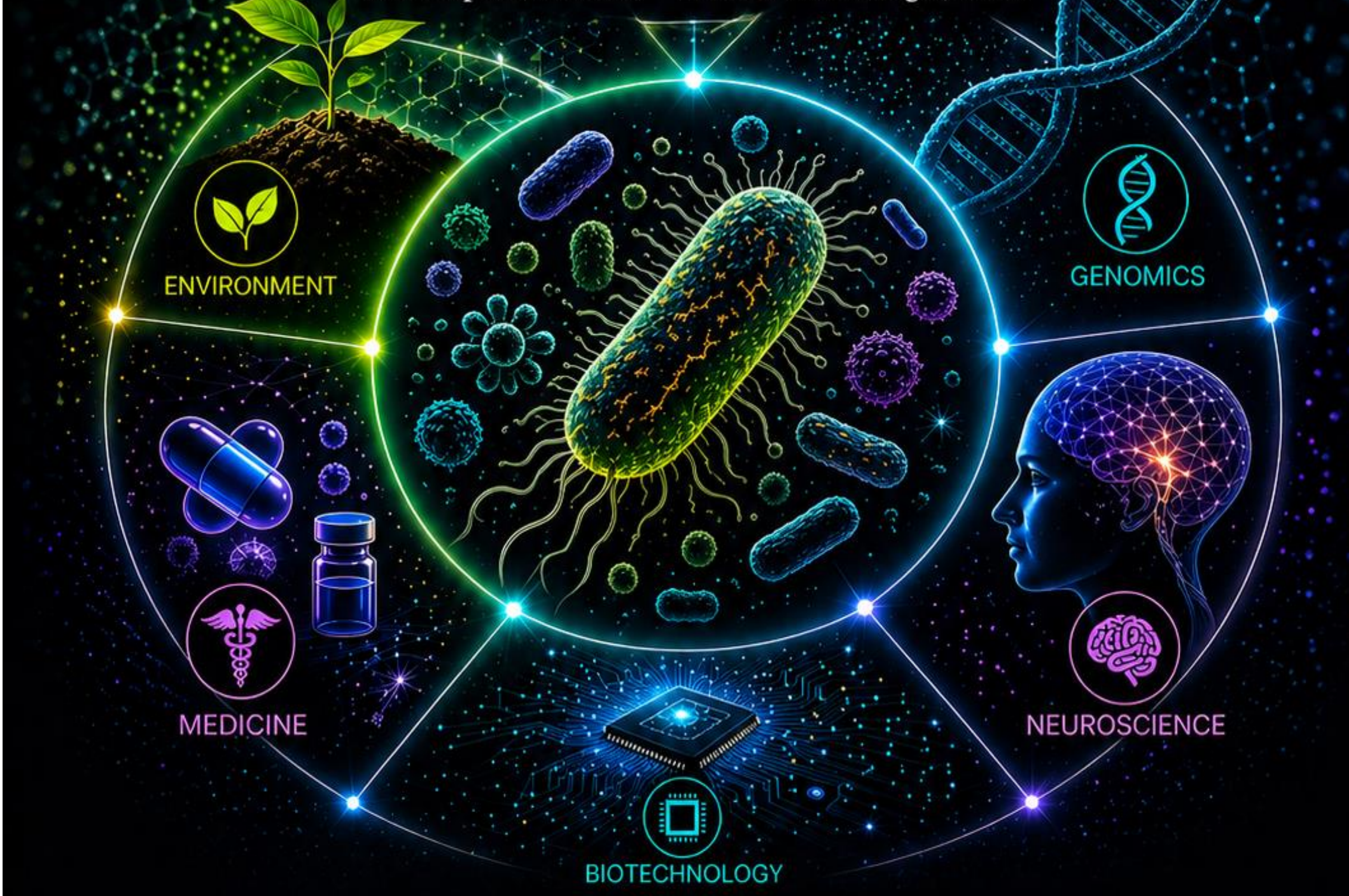

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Declaration by the Editor

I hereby declare that the book titled **“The Microbial Nexus: Intersections In Modern Science”** has been edited under my supervision. I have carefully reviewed the manuscript for clarity, coherence, structure and language and ensured that it meets the required academic and publishing standards. To the best of my knowledge, the content of this book is original and has not been published elsewhere in the same form. All sources used by the author(s) have been duly acknowledged and any necessary permissions have been obtained where applicable.

I confirm that this book is suitable for publication and dissemination.

Acknowledgments

"Na hi jñānena sadṛśaṁ pavitram iha vidyate."

"In this world, there is nothing so purifying as knowledge."

Inspired by the belief that knowledge is the highest purifier and the noblest pursuit of humankind, I humbly acknowledge with deep gratitude the individuals and institutions whose guidance, support and encouragement made this work possible. First and foremost, I would like to praise and thank God, the Almighty, who has granted countless blessing, knowledge and opportunity to accomplish the book project work. Thanks to all the authors of the various chapters for their contributions. It had been a bit of a long process from the initial outlines to developing the full chapters and then revising them in the light of reviewer's comments. I sincerely acknowledge the author's willingness to go through this process. I also acknowledge the work and knowledge of the members of our review panels, many of which had to be done at short notice. I also thank the publishing team for their professional support throughout the editorial process. My appreciation extends to colleagues and mentors in the field of Biotechnology whose guidance and discussions have enriched this work. Finally, I acknowledge the support of Swami Vivekananda University and all my colleagues for their encouragement and patience during the preparation of this book.

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Preface

The field of biotechnology has emerged as a transformative force in advancing human health, enabling the translation of fundamental biological discoveries into meaningful clinical and therapeutic applications. ***"The Microbial Nexus: Intersections In Modern Science"*** has been carefully curated to reflect this evolution, bringing together diverse perspectives that highlight the role of translational research in bridging the gap between laboratory science and patient care. As editor, it has been my objective to assemble contributions that address both the scientific depth and practical relevance of contemporary biotechnological research. The chapters in this volume explore key areas such as molecular and cellular mechanisms of disease, diagnostic and therapeutic innovations, regenerative medicine, genomics and emerging biotechnological tools that are shaping the future of healthcare. Each contribution emphasizes translational potential, underscoring pathways through which research findings can be effectively applied to improve human health outcomes. This book brings together the work of researchers, clinicians, and academicians whose collective expertise reflects the interdisciplinary nature of translational biotechnology. The contributors have not only presented current research and reviews but have also critically examined challenges related to clinical validation, ethical considerations, regulatory frameworks and accessibility of biotechnological advancements. ***"The Microbial Nexus: Intersections In Modern Science"*** is intended to serve as a valuable resource for postgraduate students, researchers, healthcare professionals and policymakers seeking insight into the dynamic landscape of translational research. By fostering dialogue across disciplines and encouraging innovation grounded in scientific rigor and social responsibility, this book aspires to contribute meaningfully to the advancement of human health.

Barrackpore, Kolkata. Aishwarya Guha

ABOUT THE EDITOR

Dr. Aishwarya Guha, currently working as Assistant Professor in the Department of Biotechnology, Swami Vivekananda University, Barrackpore, Kolkata. Dr. Guha did her Ph.D. in Cancer Immunology from Jadavpur University, Department of Life Science and Biotechnology. Her doctoral thesis focused on elucidating the role of tumor-educated platelets in promoting epithelial-to-mesenchymal transition and angiogenesis in breast cancer, alongside therapeutic interventions using aspirin. Prior to her doctoral studies, Dr. Guha completed her M.Sc. in Zoology from the University of Calcutta with a specialized focus on Molecular & Cell Biology.

Her foundational undergraduate studies were completed at Lady Brabourne College, University of Calcutta, where she earned a B.Sc. (Hons.) in Zoology and received Academic Excellence Award. This scholastic base is further highlighted by her competitive national successes, which include clearing the GATE examination in Life Sciences and securing an impressive rank in the prestigious CSIR-UGC NET (JRF + LS) examination, which earned her the CSIR JRF and SRF fellowships during her doctoral cycle.

Dr. Guha worked extensively in the field of the tumor microenvironment, cancer stem cells, tumor heterogeneity, cellular signaling, metastasis and in-vivo translational models. Her main research interests lie in tumor-educated platelets, EMT, angiogenesis and cancer stem cell-immune cell crosstalk. Dr. Guha has a robust publication record in SCI international journals, contributed to book chapters with registered ISBN numbers and has a strong presence in conference proceedings. She has presented her Ph.D. research works as a first author and co-author in 10 international and 24 national conferences, including elite gatherings like ASCO Breakthrough, ESMO Breast Cancer and the Society for Immunotherapy of Cancer (SITC).

All of her publications are in peer-reviewed and high-impact journals like *Breast Cancer Research*, *Molecular Therapy Oncology, Frontiers in Immunology*, *Frontiers in Cell and Developmental Biology*, *Molecular Cancer Research*, *Cytokine*, *Journal of Clinical Oncology* and *ESMO Open* under renowned publication houses like Springer Nature, Frontiers Media, Elsevier and the American Society of Clinical Oncology. Her research findings have drawn praise both at home and abroad, earning her the 2nd Prize in Oral Presentation at AIIMS New Delhi, alongside the prestigious SERB International Travel Grant and the CSIR Travel Grant. She is an active member of professional bodies like the American Society of Clinical Oncology (ASCO) and the European Society for Medical Oncology (ESMO).

Dr. Guha also possesses considerable peer-review and editorial acumen, regularly serving as an active reviewer for top-tier international medical journals including *Scientific Reports* (Springer Nature), *Journal of Experimental & Clinical Cancer Research* (Springer Nature), *Frontiers in Oncology*, *Frontiers in Immunology*, *Frontiers in Bioengineering and Biotechnology* and *Frontiers in Medicine*.

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Dr Bidisha Ghosh is presently serving as an Assistant Professor in the Department of Biotechnology in the School of Life Sciences, in the prestigious Swami Vivekananda University, Barrackpore. She has completed her PhD in Biophysics from Jadavpur University in 2024. Just after her Masters in Biotechnology from the Department of Life Science & Biotechnology, Jadavpur University in 2012. Dr Ghosh served as a Research Trainee in Herbicure Healthcare Bioherbal Research foundation where she continued in the role till 2017. Dr Ghosh has been working with nano materials and her thesis work entails synthesis of varied kinds of nanomaterials and exploring their potential in waste water management along with their biological applications. She has contributed to 37 Peer Reviewed Journals, 48 Book Chapters and 01 Filed Patent (National). She has attended various National and International Conferences and has won several awards in the same. Dr Ghosh has worked as a CO-PI in the ICSSR Vision Viksit Bharat 2047 Collaborative Research Project (funded by Govt. of India) entitled “Post Covid Era and the Zero Generation: Vulnerability, Resilience and Adaptive Capacity in Ageing Communities of Select Urban Spaces of North-east India”. Dr Ghosh is a member of Society of Conservation Biology.

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Dr. Suranjana Sarkar holds a Bachelor's, Master's, and Ph.D. degree in Microbiology. Her doctoral research was centered on Drug Discovery and Rational Drug Design. Her research is fundamentally grounded in microbiology, with emphasis on microbial pathogenesis, antimicrobial strategies, and the development of therapeutics targeting infectious diseases. She has published in peer-reviewed journals, and her ongoing research interests focus on combinational antimicrobial systems, comparative evaluation of natural and synthetic therapeutics, and systems biology–driven drug–target interaction and safety profiling along with ML-assisted quantum chemical computational based drug designing. Dr. Sarkar is committed to advancing microbiology-driven therapeutic innovation and mentoring emerging researchers in interdisciplinary life sciences. At Swami Vivekananda University, she teaches both Undergraduate and Postgraduate courses in Biotechnology and Microbiology, delivering core and applied subjects including *General Microbiology, Microbial Genetics, Biochemistry, Quality Assurance and Quality Control, and Medical Coding & Scribing*. She integrates foundational microbiology with translational and industry-oriented perspectives in her teaching. She is committed to student mentorship, providing personalized academic guidance and professional development support to foster students' holistic growth and research excellence. She effectively balances research and academics, aiming excellence in both scientific inquiry and student learning.

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Dr. Subhasis Sarkar, an Assistant Professor in Microbiology of Swami Vivekananda University, has completed his Ph.D in Biotechnology from University of Calcutta in 2014. After completing this journey, he served the department of Molecular biology & Bioinformatics, Tripura University (A Central University) as Guest Faculty, from September, 2014 till May, 2015. Thereafter, he contributed as Senior Project Fellow at the Institute of Environmental Studies and Wetland Management till 2016. Later on, he served as Assistant Professor in Microbiology in Kingston College of Science together with has served as Guest Faculty in Panihati College and West Bengal State University in various department including Food & Nutrition and Microbiology. In the post PhD journey of 12 Years, he has guided UG, PG and PhD students in different relevant domains of Microbiology and Biotechnology. Till date he has contributed in various in 46 articles in Book Chapters, 51 articles in Peer Reviewed Journals and 01 patent (National). Right Now, his work has been appreciated as evidenced in Google scholar record of 2108 citations with H index 12 and i-10 index 13. During presenting his work in National and International conferences, he has been awarded twice with 2nd Prize (Speaker) in National level Platform. He has been recognized by a couple of prestigious editorial board as reviewer including 3-Biotech, Frontiers in Pharmacology etc. He has been serving as a member of American Society for Microbiology, Society for Conservation Biology (USA) etc.

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His lab currently focusing on Drug Discovery, Protein Engineering, and Mechanistic Toxicological Assessment of compounds integrating both computational and experimental approaches. His work exploring natural and synthetic bioactive molecules, engineers' proteins and enzymes with therapeutic relevance, and evaluates safety through toxicity profiling in biological models. Dr. Banerjee has contributed substantially to the field through publications in leading journals, including Science Advances, Molecular Aspects of Medicine, EBioMedicine, Frontiers in Immunology etc. Beyond research, he is deeply dedicated to teaching and mentorship, inspiring the next generation of life sciences students and researchers at Swami Vivekananda University to pursue transformative scientific discoveries.

CHAPTER 1: Bioaccumulation of Arsenic in Ginger (Zingiber officinale): Sources, Mechanisms, and Health Impacts

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Abstract: Arsenic, a toxic metalloid, is becoming a major contaminant in agroecosystems—particularly across South and Southeast Asia. Its widespread presence in groundwater and soil threatens both crops and public health. Ginger (Zingiber officinale), a widely consumed rhizome valued for its medicinal and economic significance, has shown notable potential for arsenic accumulation. This review examines how arsenic enters ginger, the plant tissues where it accumulates, and the cellular and molecular mechanisms underlying arsenic toxicity. Groundwater used for irrigation is the main source of arsenic contamination in agricultural soils, from which arsenic is absorbed by roots and translocated to the edible rhizome. Research indicates arsenic primarily accumulates in the cortical tissues of the rhizome, where it disrupts cell structure by oxidative stress, DNA damage, and enzyme inhibition. These toxic problems can compromise plant health and present dietary risks to populations consuming contaminated ginger. The review also discusses patterns of disease prevalence linked to arsenic exposure and includes visual pathways summarizing arsenic movement, toxicity mechanisms, and related diseases. The findings highlight the urgent need for effective regulatory and remediation strategies to limit arsenic uptake in ginger and ensure food safety.

Keywords: Arsenic bioaccumulation, Zingiber officinale, Heavy metal toxicity, Arsenic transport, Public Health.

1. Introduction

Arsenic is a naturally occurring metalloid present in the Earth's crust. Human activities such as mining, the use of arsenic-based pesticides, and irrigation with contaminated groundwater have significantly increased its release into the environment (Sharma et al., 2021). In areas where groundwater is the main source of irrigation—such as West Bengal, Bihar, and Bangladesh—arsenic concentrations routinely exceed safe limits, entering agricultural soils and, subsequently, food crops (Sarkar, Paul, & Majumdar, 2022). Ginger (Zingiber officinale), a globally traded crop with culinary and medicinal applications, is increasingly recognized for its tendency to bioaccumulate arsenic (Das et al., 2023).

Several factors influence arsenic bioaccumulation in ginger, including:

- Soil composition, redox potential, chemical metrics, and soil-layer architecture.

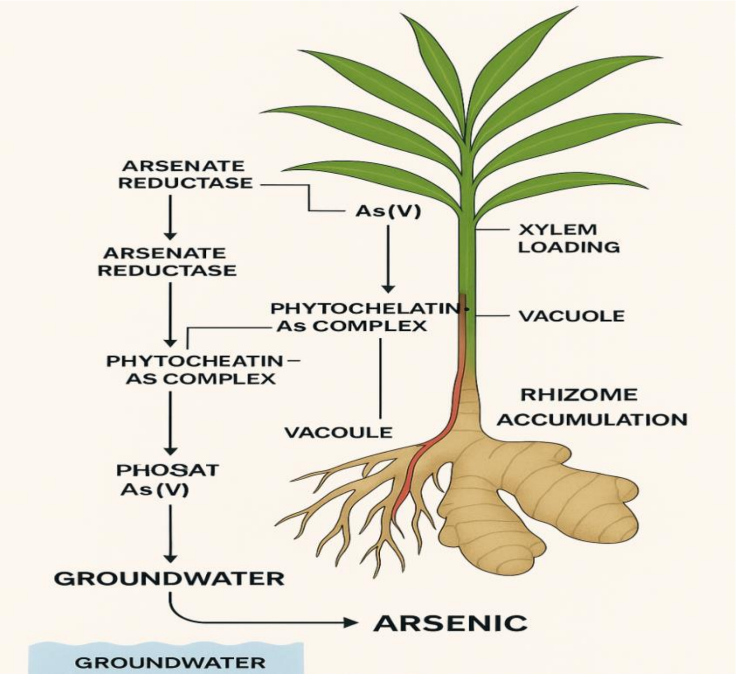
- Local irrigation practices and absolute reliance on contaminated groundwater pools.
- The plant's direct genetic and physiological capacity for selective arsenic uptake and vascular translocation.

Research indicates that arsenic tends to concentrate in specific tissues such as roots and rhizomes—the principal edible parts of ginger. This situation raises significant food safety concerns, as chronic exposure to arsenic, even at low concentrations, is associated with carcinogenic, neurotoxic, and developmental health risks.

The uptake and movement of arsenic from environmental sources—groundwater and contaminated soils—into ginger involve complex biochemical and physiological mechanisms. These processes affect plant growth and yield while also triggering oxidative stress pathways and disrupting essential cellular functions. Epidemiological studies have linked chronic arsenic exposure to various diseases, with distinctive patterns of prevalence observed regionally and globally.

2. Arsenic Entry Pathways: Groundwater to Ginger

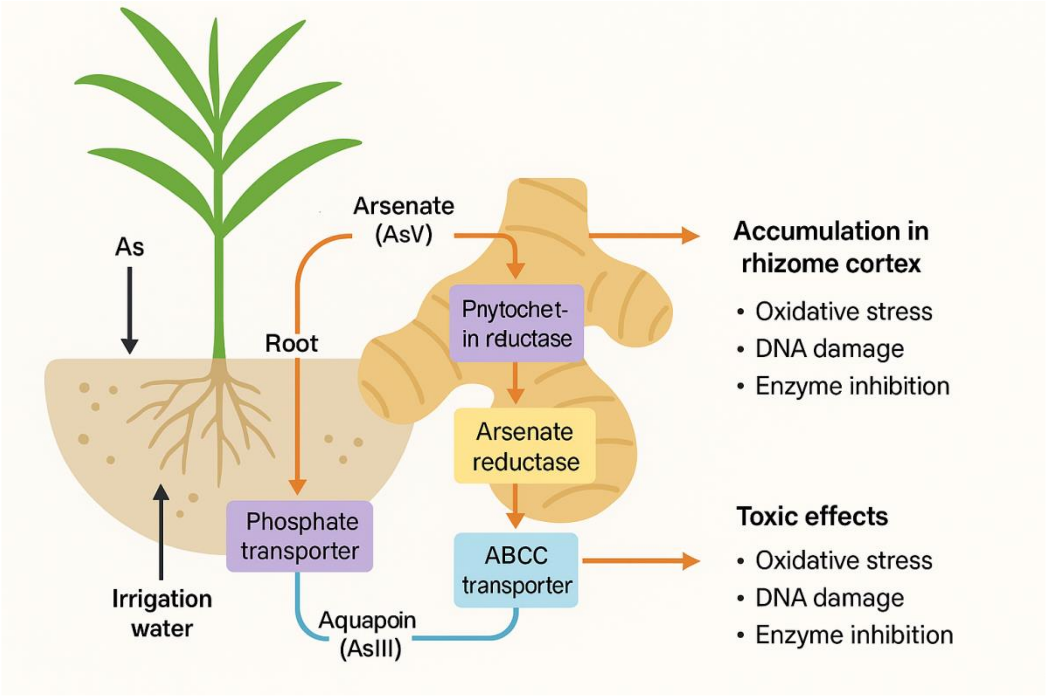
The contamination process typically starts with the use of rich arsenic groundwater for irrigation. Once it is introduced into the soil, arsenic is found primarily as arsenate (As5+) or arsenite (As3+). For its structural similarity to phosphate, arsenate enters plant roots via phosphate transporters (Kumar et al., 2020). Once arsenic is in the root system, it can be taken up actively or passively, and is then transported through the xylem to above-ground parts, including the rhizome (Kumar et al., 2020). Factors such as soil pH, redox potential, and the presence of iron and organic matter strongly influence arsenic mobility and uptake.



3. Plant Tissues of Arsenic Accumulation

Research and ICP-MS studies reveal that arsenic mostly accumulates in the cortical and vascular tissues of the ginger rhizome (Patel et al., 2021). Rhizomes show higher arsenic concentrations when compared to roots and leaves, showing direct exposure risks to consumers.

The physical accumulation follows the hierarchical trend: Root > Rhizome > Leaf, with the highest systemic accumulation in outer cortical tissues where metabolic activity is structurally high (Das et al., 2023). Long-term cultivation in contaminated soils exacerbates this tissue-specific buildup over time.



4. Cellular and Molecular Toxicity in Ginger

Arsenic disrupts normal physiological functions at both the cellular and molecular levels, creating a dual-layered toxicity profile:

4.1 Cellular Disruption: At the cellular layer, arsenic induces extensive lipid peroxidation and severe membrane damage through the chronic overproduction of reactive oxygen species (ROS) (Saha & Mandal, 2020). This cascade triggers clear phenotypic anomalies including chlorosis, significantly reduced rhizome mass, compromised photosynthesis, and compromised crop quality (Patel et al., 2021).

4.2 Molecular Inactivation: Molecularly, it directly interferes with DNA replication and repair structural configurations, inhibits critical enzymes like ATPases, and causes severe protein misfolding (Kumar et al., 2020). Arsenic stress also significantly alters the active expression profiles of antioxidant enzymes such as catalase and superoxide dismutase, further compromising the plant's baseline immune defense mechanisms (Saha & Mandal, 2020).

5. Human Health Impact: Disease Prevalence

The ingestion of arsenic-contaminated ginger leads to prolonged heavy metal exposure in humans. Prolonged consumption may lead to severe dermal lesions (hyperkeratosis and advanced pigmentation changes), peripheral neuropathy, cardiovascular conditions (blackfoot disease, severe hypertension), and advanced carcinogenesis (Mukherjee & Chatterjee, 2021). Epidemiological studies consistently associate dietary arsenic exposure with a heightened risk of internal and external malignancies, notably of the skin, bladder, lungs, and liver (Sarkar et al., 2022).

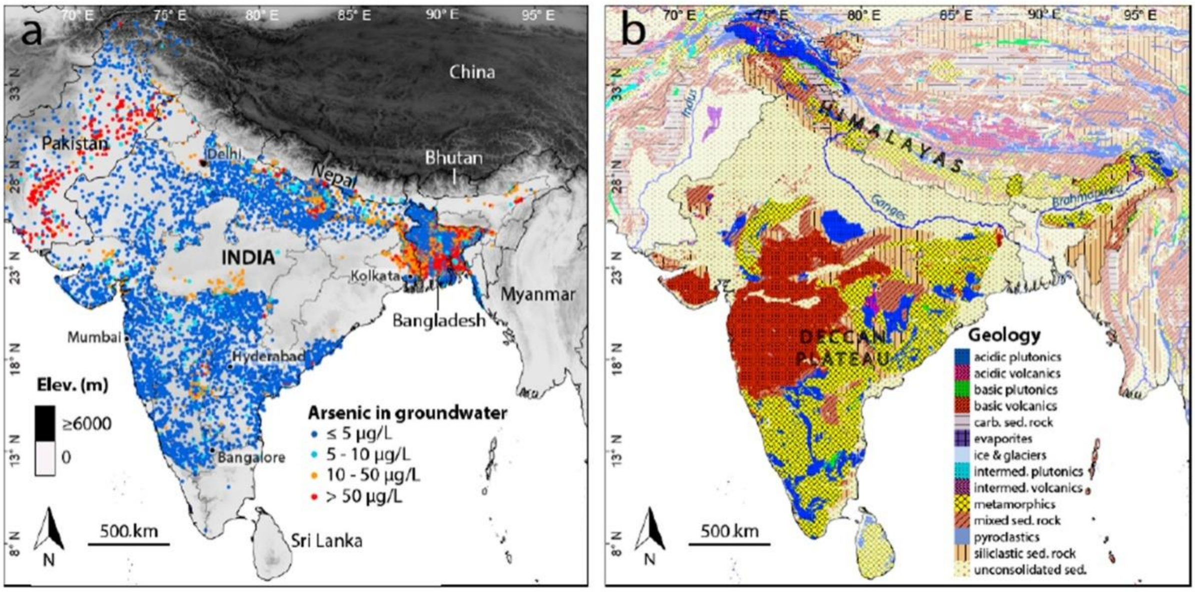
Globally, more than 140 million individuals are projected to be at risk from arsenic in potable water and food matrices (WHO, 2020). In India, arsenic-related disorders are primarily documented in the Indo-Gangetic region, particularly across West Bengal, Assam, Bihar, and Uttar Pradesh (Mukherjee & Chatterjee, 2021).

6. Disease Prevalence Related to Arsenic Exposure

REGION	COMMON DISEASES	SOURCE OF EXPOSURE
West Bengal (India)	Skin lesions, hyperkeratosis, cancer, peripheral neuropathy	Drinking water, contaminated root/rhizome crops
Bangladesh	Hepatocellular, bladder, and lung carcinomas	Groundwater irrigation, dietary vegetables
Global (e.g., USA, China)	Bladder cancer, cardiovascular disease, systemic diabetes	Arsenic-contaminated food streams, processed grains

7. Conclusion

The bioaccumulation of arsenic in Zingiber officinale is a growing concern due to the increasing contamination of groundwater and agricultural soils, particularly in South and Southeast Asia. As a widely consumed medicinal and dietary plant, ginger's contamination with arsenic directly threatens food safety, agricultural sustainability, and public health. This review has clarified the major routes of arsenic transport from contaminated groundwater into the rhizosphere and then into ginger's edible rhizomes. Studies confirm that the highest arsenic accumulation occurs in the root tissues, especially the cortex and vascular tissues, which serve as primary sinks due to high surface contact and metabolic activity.



Arsenic induces toxicity through mechanisms affecting both cellular and molecular levels. It disturbs cellular homeostasis by obstructing water channels, phosphate transporters, and enzymatic systems, including ATP synthase and antioxidative enzymes. Arsenite [As(III)] specifically binds to sulfhydryl groups in proteins, resulting in oxidative stress, mitochondrial dysfunction, and programmed cell death (apoptosis) in plant cells. Molecular-level alterations hinder plant growth, photosynthesis, and biomass productivity, hence directly affecting the yield and quality of ginger rhizomes.

The human health impacts of arsenic-laden ginger and other food crops are far-reaching. Chronic arsenic exposure has been linked to various carcinogenic and non-carcinogenic outcomes, including skin lesions, cardiovascular disease, diabetes, neurological disorders, and cancers of the bladder, lungs, and skin. In India, regions like West Bengal, Bihar, Assam, and Uttar Pradesh report alarmingly high groundwater arsenic levels, making populations in these areas especially vulnerable. Globally, Bangladesh, China, and parts of South America face similar issues, signifying that arsenic bioaccumulation in crops is a pressing international concern.

The data highlight an urgent need for integrating site-specific phytoremediation strategies, improved irrigation practices, and the use of arsenic-resistant ginger cultivars. Equally important are awareness campaigns and policy-level interventions to monitor and regulate arsenic levels in both agricultural environments and commercial plant products. Modern techniques such as arsenic speciation analysis, transcriptomic profiling of stress-response genes in ginger, and genome editing to enhance detoxification capacity could provide long-term, sustainable solutions.

In summary, the issue of arsenic bioaccumulation in Zingiber officinale is not merely an environmental or agronomic problem—it is a multidimensional threat spanning agriculture, human health, and environmental justice. Future research must bridge molecular biology with ecological and public health frameworks to develop a comprehensive, transdisciplinary approach for detection, prevention, and mitigation of arsenic contamination in staple and medicinal crops.

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CHAPTER 2: Enzymes as Biopharmaceuticals: Recent Developments and Therapeutic Potential

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Abstract: Enzymes are normal proteins that accelerate chemical reactions within the body. In recent times, they have been given prominence as powerful biopharmaceuticals to treat numerous diseases. This article highlights the recent progress in enzyme-based therapies and indicates their growing role in modern medicine. Enzyme biopharmaceuticals are presently used to treat various diseases, including genetic disorders, cancer, infections, and metabolic syndromes. For example, enzyme replacement therapy (ERT) has been effectively used to treat such disorders as Gaucher's disease, Fabry disease, and Pompe disease. Enzymes are also on the list for cancer treatment to break down the tumor shield barrier or activate cancer medication within the tumor site. In infections, certain enzymes can lyse biofilms, which will make antibiotics more effective. Enhanced stability, targeting, and activity of enzymes have been realized with advanced biotechnology, such as genetic engineering and nano-delivery systems. A few challenges still remain, such as high production cost, immune response, and restricted delivery to target tissues. Researchers today are trying to come up with novel methods to overcome such limitations, such as using engineered enzymes, encapsulation, and intelligent delivery systems. Such a review given here is an overview of how enzymes are being used as biopharmaceuticals, the diseases they can treat, developments thus far, and where an exciting field is going. With research and development ongoing, enzyme-based therapies have the potential to be safer, less expensive, and more accessible in the health care field.

Keywords: Enzymes, Pharmaceuticals, Encapsulation, Delivery system, Enzyme based therapy

1. Introduction

Enzymes as biopharmaceuticals represent a rapidly advancing frontier in modern medicine, offering highly specific and efficient therapeutic interventions for a wide range of diseases. As biological catalysts, enzymes possess remarkable substrate specificity and catalytic efficiency, enabling them to modulate biochemical pathways with precision. Their application as therapeutic agents has grown significantly within Biopharmaceutical science, driven by advances in molecular biology, protein engineering, and drug delivery systems. Historically, enzymes were primarily utilized in industrial and diagnostic settings; however, their transition into clinical therapeutics has transformed the management of conditions such as metabolic disorders, cancer, and cardiovascular diseases.

One of the earliest and most successful applications of enzyme-based therapy is enzyme replacement therapy (ERT), which has been widely used to treat lysosomal storage disorders. These inherited metabolic conditions arise from deficiencies in specific enzymes, leading to the accumulation of toxic substrates within cells. By administering recombinant forms of the missing enzymes, ERT restores metabolic function and alleviates disease symptoms. Clinical success in disorders such as Gaucher disease and Fabry disease has demonstrated the therapeutic potential of enzymes, although limitations such as high cost, immune responses, and restricted tissue distribution remain significant challenges.

Recent developments in protein engineering have significantly expanded the scope of enzyme therapeutics. Techniques such as rational design and directed evolution enable the modification of enzyme structure to enhance stability, activity, and specificity. These approaches have facilitated the development of enzymes with improved pharmacokinetic properties, including extended half-life and reduced immunogenicity. For example, chemical modifications such as PEGylation and fusion to long-circulating proteins have been employed to prolong systemic exposure and reduce the frequency of dosing. Additionally, advances in computational modeling and artificial intelligence are increasingly being used to predict protein folding and optimize enzyme function, accelerating the development pipeline.

Another important area of innovation is targeted enzyme delivery. One of the major barriers to effective enzyme therapy is the inability of enzymes to reach specific tissues or cross biological barriers such as the blood-brain barrier. To address this, researchers have developed various delivery platforms, including nanoparticles, liposomes, and antibody-enzyme conjugates. These systems not only protect enzymes from degradation but also enable site-specific delivery, thereby enhancing therapeutic efficacy while minimizing off-target effects. In oncology, strategies such as enzyme-directed prodrug therapy have shown promise in selectively activating cytotoxic agents at tumor sites, reducing systemic toxicity.

Enzyme therapeutics have also shown significant potential in the treatment of cancer. Certain enzymes can directly target tumor metabolism or modulate the tumor microenvironment. For instance, L-asparaginase is used in the treatment of acute lymphoblastic leukemia by depleting circulating asparagine, an amino acid essential for tumor cell survival. Similarly, uricase-based therapies have been employed to manage hyperuricemia associated with tumorlysis syndrome and refractory gout. These examples highlight the versatility of enzymes in addressing diverse pathological conditions through distinct mechanisms of action.

Despite these advances, several challenges continue to limit the widespread adoption of enzyme-based therapies. Immunogenicity remains a major concern, as exogenous enzymes can trigger immune responses that reduce efficacy and cause adverse effects. Strategies such as protein humanization, epitope removal, and immune tolerance induction are being explored to mitigate these risks. Additionally, the high cost of enzyme production and purification poses economic barriers, particularly in low-resource settings. Manufacturing complexities, including maintaining protein stability and ensuring batch-to-batch consistency, further complicate large-scale production.

Regulatory considerations also play a critical role in the development of enzyme biopharmaceuticals. Ensuring safety, efficacy, and quality requires rigorous preclinical and clinical evaluation. Advances in clinical trial design, including adaptive trials and real-world evidence integration, are helping to streamline the approval process while maintaining high standards. Furthermore, the emergence of personalized medicine is influencing the development of enzyme therapies, with treatments increasingly tailored to individual genetic and molecular profiles.

Looking ahead, the future of enzymes as biopharmaceuticals is highly promising. Innovations in gene therapy and mRNA-based technologies are opening new avenues for in vivo enzyme production, potentially overcoming limitations associated with repeated dosing. Genome editing tools such as CRISPR-Cas systems may enable permanent correction of enzyme deficiencies at the genetic level. Additionally, the integration of nanotechnology and synthetic biology is expected to further enhance enzyme stability, delivery, and functionality.

2. Therapeutic Enzyme Classes and Approved Use Cases

Therapeutic enzymes represent a major category of biologic drugs within Biopharmaceutical science, offering targeted treatment for metabolic, hematologic, oncologic, and rare genetic disorders. They are broadly classified into enzyme replacement therapies, thrombolytic enzymes, metabolic-depleting enzymes, and detoxifying enzymes, each with distinct approved clinical applications.

Enzyme replacement therapy (ERT) is one of the most established uses of therapeutic enzymes. It is primarily used to treat lysosomal storage disorders caused by inherited enzyme deficiencies. Recombinant enzymes are administered to restore missing metabolic activity and reduce substrate accumulation. Approved examples include imiglucerase for Gaucher disease and agalsidase beta for Fabry disease. These therapies improve organ function, reduce disease progression, and enhance quality of life, although lifelong treatment is typically required.

Thrombolytic enzymes are another clinically important class. Agents such as tissue plasminogen activator (tPA) are used to treat acute ischemic stroke, myocardial infarction, and pulmonary embolism. tPA works by converting plasminogen into plasmin, which degrades fibrin clots and restores blood flow. Its rapid action makes it a critical intervention in time-sensitive cardiovascular emergencies.

Metabolic-depleting enzymes are widely used in oncology. A key example is L-asparaginase, which is approved for the treatment of acute lymphoblastic leukemia. It depletes circulating asparagine, an amino acid essential for lymphoblast survival, thereby selectively inhibiting tumor growth while sparing most normal cells. This targeted mechanism has made it a cornerstone in pediatric leukemia therapy.

Detoxifying enzymes also play an important therapeutic role. Uricase enzymes, such as pegloticase, are used in refractory gout to catalyze the conversion of uric acid into allantoin, a more soluble compound. This is particularly beneficial for patients who do not respond to conventional urate-lowering drugs. Similarly, other enzyme systems are being explored for detoxification of harmful metabolites in metabolic disorders.

Despite their clinical success, therapeutic enzymes face several limitations. Immunogenic reactions can reduce efficacy and cause adverse effects, particularly with repeated administration. Additionally, short plasma half-life and poor tissue targeting limit their effectiveness. Advances in PEGylation, glycoengineering, and nanoparticle-based delivery systems are helping overcome these challenges by improving stability and pharmacokinetics.

Overall, therapeutic enzymes have transformed the management of multiple diseases by providing highly specific and mechanism-based interventions. Ongoing research in protein engineering and drug delivery is expected to expand their clinical applications further and improve patient outcomes.

3. Recent Innovations in Enzyme Engineering and Delivery

3.1 Protein Engineering and Directed Evolution

Protein engineering and directed evolution are transformative approaches in Biotechnology that enable the design and optimization of proteins with enhanced or novel functions. Protein engineering typically involves rational design, where structural and functional knowledge guides targeted modifications to amino acid sequences. In contrast, directed evolution mimics natural selection by generating large libraries of protein variants through mutagenesis and recombination, followed by screening or selection for desired traits.

Directed evolution has been widely applied to improve enzyme activity, stability, and substrate specificity, often achieving results unattainable by rational design alone. A landmark example is the work of Frances H. Arnold, who pioneered the use of iterative mutation and selection to evolve enzymes with enhanced catalytic properties, earning the Nobel Prize in Chemistry in 2018. These methods are now central to industrial biocatalysis, drug development, and synthetic biology. Despite their success, challenges include the need for efficient screening techniques and the complexity of protein folding and function. Integration of computational tools with experimental evolution is expanding the scope and precision of protein engineering.

3.2 Half-Life Extension and Immunogenicity Reduction

Half-life extension and immunogenicity reduction are key goals in the design of therapeutic proteins and biologics within Biopharmaceutical science. Many protein drugs have short circulation times due to rapid renal clearance and proteolytic degradation. Strategies to extend half-life include PEGylation (attachment of polyethylene glycol chains), fusion to long-lived proteins such as albumin or Fc fragments of antibodies, and glycoengineering. For example, PEGylation increases molecular size and shields proteins from enzymatic breakdown, thereby prolonging systemic exposure.

Immunogenicity, the ability of a therapeutic protein to trigger an immune response, can reduce efficacy and cause adverse effects. Approaches to minimize immunogenicity include humanization of monoclonal antibodies, removal of T-cell epitopes, and optimization of protein folding to avoid aggregation. Advances in computational prediction tools have improved the identification of immunogenic regions, enabling rational design of safer biologics. Balancing extended half-life with reduced immunogenicity remains challenging, as modifications may alter protein structure and function. Ongoing research integrates protein engineering and immune profiling to develop next-generation biologics with improved safety and efficacy.

3.3 Overcoming Biological Barriers

Overcoming biological barriers is a central challenge in enzyme technology, particularly for therapeutic and industrial applications within Biotechnology. Enzymes administered in vivo must navigate barriers such as proteolytic degradation, immune recognition, cellular membranes, and restricted tissue access, including the blood-brain barrier. These obstacles can significantly limit enzyme stability, bioavailability, and efficacy.

Various strategies have been developed to address these challenges. Encapsulation techniques, such as liposomes and polymeric nanoparticles, protect enzymes from degradation and enhance targeted delivery. Chemical modifications like PEGylation improve enzyme stability and circulation time. Additionally, protein engineering enables the design of enzymes with enhanced resistance to harsh physiological conditions. Targeting ligands, such as antibodies or receptor-specific peptides, can facilitate uptake into specific cells or tissues, improving therapeutic precision. Crossing the blood-brain barrier remains particularly difficult; however, approaches such as receptor-mediated transcytosis and fusion with transport proteins show promise. Despite these advances, issues such as immunogenicity, manufacturing complexity, and cost persist. Continued integration of nanotechnology, molecular biology, and computational design is driving innovation in overcoming biological barriers, enabling more effective enzyme-based therapies and industrial biocatalysts.

3.4 Nano-Enabled Enzyme Delivery

Nano-enabled enzyme delivery is an emerging strategy in Nanomedicine that enhances the stability, targeting, and therapeutic efficacy of enzymes. Enzymes are highly specific biocatalysts but often suffer from rapid degradation, poor cellular uptake, and limited bioavailability in physiological environments. Nanocarriers such as liposomes, polymeric nanoparticles, dendrimers, and inorganic nanomaterials are designed to encapsulate or conjugate enzymes, protecting them from proteolysis and extending circulation time.

These nanosystems can be functionalized with targeting ligands to enable site-specific delivery, improving therapeutic precision while reducing off-target effects. For example, enzyme-loaded nanoparticles have shown promise in treating cancer, neurodegenerative disorders, and lysosomal storage diseases by facilitating intracellular delivery and, in some cases, transport across biological barriers like the blood-brain barrier. Additionally, stimuli-responsive nanocarriers can release enzymes in response to pH, temperature, or redox conditions, enhancing controlled delivery. Despite these advantages, challenges remain, including potential nanotoxicity, large-scale manufacturing, and regulatory hurdles. Continued advances in material science and protein engineering are expected to further optimize nano-enabled enzyme delivery systems.

4. Clinical Evidence across Indications

Clinical evidence across indications is essential for evaluating the safety and efficacy of therapies within Clinical research. Evidence is typically generated through randomized controlled trials (RCTs), observational studies, and real-world data analyses, each contributing unique insights into treatment performance. RCTs remain the gold standard for establishing efficacy and causality, while real-world evidence helps assess effectiveness in broader, more diverse patient populations.

Across multiple disease areas such as oncology, cardiovascular disorders, and rare diseases clinical evidence supports regulatory approval and informs clinical guidelines. For instance, therapies validated in one indication may be investigated for others through adaptive trial designs or drug repurposing strategies. This cross-indication evaluation is particularly important in personalized medicine, where biomarkers guide treatment selection across different conditions. However, challenges include heterogeneity in patient populations, variability in study endpoints, and differences in trial design. Integrating data from multiple sources requires robust statistical methods and standardized reporting practices. Regulatory agencies increasingly emphasize comprehensive evidence packages that combine clinical trial data with real-world outcomes. Overall, synthesizing clinical evidence across indications enhances decision-making, supports broader therapeutic applications, and improves patient care.

5. Safety and Translational Challenges

The clinical utility of therapeutic enzymes is constrained by several persistent challenges. Immunogenicity remains a primary barrier, often precipitating loss of function and adverse infusion reactions. Furthermore, suboptimal biodistribution and abbreviated half-lives compromise systemic efficacy, necessitating the development of advanced delivery mechanisms such as nanocarriers, fusion proteins, and sustained-release systems.

A critical bottleneck is the blood-brain barrier (BBB), which leaves the neurological manifestations of lysosomal storage diseases largely untreatable, though emerging strategies like receptor-mediated transcytosis and in utero interventions provide promising pathways. Finally, high production and administration costs underscore the urgent need for more resilient, gene-enabled, or orally available enzyme platforms to broaden patient access.

6. Future Directions

The future of enzymes as biopharmaceuticals within Biopharmaceutical science is being shaped by rapid advances in molecular engineering, delivery technologies, systems biology, and precision medicine. Therapeutic enzymes have already demonstrated clinical success in enzyme replacement therapies, cancer treatment, and thrombolysis, but their full potential is still emerging. Future developments are expected to focus on improving efficacy, reducing immunogenicity, expanding target accessibility, and enabling personalized treatment strategies.

A major direction of innovation is next-generation protein engineering. Traditional enzyme therapeutics often face limitations such as instability, short half-life, and immune recognition. Modern approaches such as directed evolution and computational protein design allow precise optimization of enzyme structure and function. Machine learning models are increasingly used to predict protein folding, stability, and substrate specificity, significantly accelerating enzyme development cycles. These technologies are expected to produce highly stable, human-compatible enzymes with improved catalytic efficiency and reduced immunogenicity.

Another promising direction is gene- and RNA-based enzyme delivery. Instead of repeated enzyme infusions, messenger RNA (mRNA) and gene therapy platforms can enable in vivo production of therapeutic enzymes. This approach has gained momentum following advances in lipid nanoparticle (LNP) delivery systems. By introducing genetic instructions rather than proteins themselves, long-term or even permanent enzyme expression may be achieved, particularly for metabolic diseases. However, challenges such as controlled expression levels and long-term safety must be addressed. Targeted delivery systems are also expected to transform enzyme therapeutics. One of the major limitations of current enzyme drugs is their inability to reach specific tissues, especially across biological barriers such as the blood–brain barrier. Nanotechnology-based delivery systems, including liposomes, polymeric nanoparticles, and exosome-mimetic vesicles, are being developed to improve tissue-specific targeting. These systems can protect enzymes from degradation and enable controlled release in diseased tissues. Functionalization with antibodies or ligands further enhances selectivity, which is particularly important in oncology and neurological disorders.

The integration of enzyme therapy with precision medicine is another important future direction. Advances in genomics and biomarker discovery now allow diseases to be stratified at the molecular level. This enables the selection of enzyme therapies tailored to individual genetic profiles. For example, patients with lysosomal storage disorders may benefit from genotype-specific enzyme variants with improved uptake or reduced immunogenicity. Such personalized approaches are expected to increase treatment efficacy and reduce adverse effects.

Combination therapies involving enzymes are also gaining attention. Enzymes can be used alongside small-molecule drugs, monoclonal antibodies, or immune checkpoint inhibitors to achieve synergistic effects. In oncology, enzyme-directed prodrug therapy (GDEPT and ADEPT) is being explored to selectively activate cytotoxic agents within tumor microenvironments, minimizing systemic toxicity. These strategies are expected to expand the applicability of enzyme therapeutics in solid tumors and resistant cancers.

Immunogenicity remains a critical challenge for future enzyme therapeutics. Even highly purified recombinant enzymes can trigger immune responses that reduce efficacy or cause hypersensitivity reactions. Future solutions include epitope editing, human protein scaffolding, immune tolerance induction, and stealth coating strategies such as PEGylation. Advances in immunoinformatics are also enabling better prediction of T-cell epitopes, supporting the design of less immunogenic enzymes.

Finally, emerging fields such as synthetic biology and systems biotechnology are expected to redefine enzyme therapeutics. Synthetic gene circuits can be engineered to convert dynamic physiological parameters into localized therapeutic actions, enabling smart therapeutics that activate exclusively inside microscale pathological environments.

7. Conclusions

In conclusion, enzymes have emerged as a powerful and versatile class of biopharmaceuticals, offering highly specific and efficient therapeutic interventions across a wide range of diseases. Recent developments in protein engineering, recombinant DNA technology, and drug delivery systems have significantly enhanced the stability, efficacy, and targeting capabilities of enzyme-based therapies. These advances have expanded their clinical applications beyond traditional uses, enabling innovative treatments for genetic disorders, cancer, infectious diseases, and metabolic conditions. Despite ongoing challenges such as immunogenicity, short half-life, and high production costs, continued research and technological innovation are steadily addressing these limitations. Overall, the growing integration of enzymes into modern therapeutics underscores their immense potential, positioning them as a cornerstone in the future of precision medicine and next-generation biopharmaceutical development.

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CHAPTER 3: ZIKA virus as an oncolytic adenovirus in malignant glioma preclinical evidence, engineering status and challenges in translation

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Abstract

Malignant gliomas, specifically glioblastoma (GBM) show a poor prognosis and make an oncolytic virotherapy (OV) an effective therapy strategy alternative. The reason that makes the Zika virus (ZIKV) a unique candidate oncolytic virus is its unique capability and inherent tropism to infect nerve progenitor-like cells in which such neoplasms proliferate.

This study summarizes the preclinical findings which show that the ZIKV, selective to glioma stem cells (GSCs), kills ZIKV which in turn causes significant tumor remission and prolongs survival in mice and dogs. Besides, ZIKV therapy reprograms the immunosuppressive tumor microenvironment making it an immunologically cold, but hot, and provide lasting anti-tumor immunity.In response to campaign clinical safety issues, recent technology in the field of genetic engineering has brought about microRNA-sensorable versions of ZIKV that demonstrate agwam replication in unaffected neural tissue who nevertheless maintain oncolytic activity. Such results make ZIKV, and particularly its genetically engineered analogs, a powerful and highly versatile oncolytic platform that can be translated to the clinic to deal with malignant tumors in the brain.

Keywords: Anti-tumor immunity, Glioma stem cells (GSCs), MicroRNA, Oncolytic virotherapy (OV), Zika virus (ZIKV).

1. Introduction

Malignant gliomas, specifically glioblastoma (GBM) are among the deadliest malignancies, with the median overall survival not much more than two years, despite the utilization of multiple aggressive treatments of care (Coats et al, 2025, Calderón-Peláez et al., 2024). Failure to respond to treatment can be explained by an active invasiveness of the tumour, the high level of heterogeneity with respect to cells, a deep level of immunosuppression in the microenvironment, and the protection when exposed to the blood-brain barrier (BBB), reducing the efficacy of the majority of the systemic pharmacotherapies significantly.

Oncolytic virotherapy (OV) has become a new approach form of therapeutics that directly solves these problems. This mode utilizes viruses, which target and infect malignant cells selectively inducing lysis in them with the concomitant response of a strong anti-tumor immune reaction against them (Ferreira et al, 2024). Oncolytic viruses have the ability to reshape a warm hot phenotype of the immunologically cold microenvironment of glioma through the release of a cascade of tumour antigens and pro-inflammatory cytokines, which exposes the therapeutic target to immune-mediated attack (Coates et al., 2025). The clinical practicability of such a method is proven with a number of viral vectors, such as HSV -1, adenovirus, and the proprietary oncolytic HSV -1 virus G47Δ- being taken through clinical trials; G47Δ has conditional clinical trial approval in Japan, in the treatment of glioma (Yajimam, et al., 2021). The Zika virus (ZIKV) as a potential oncolytic virus has its own natural unique advantages. It is neuropathogenic and has a strong intrinsic affinity to the glioma stem cells (GSCs) (Iannolo et al., 2019; Zhu et al., 2017), close phenotypically and functionally related to the neural progenitor cells (NPC): the neuritogen cadherins of selective microcephaly, that are centrally involved in tumour growth and recurrence. This anti-glutabalanin affinity faces ZIKV to eliminate GSCs and spares the mature, differentiated neural tissue to a large extent. Preclinical models have continued to show that ZIKV suppresses tumour growth in murine models, specifically, that it induces tumour remission and neurological rescues in canine subjects with spontaneously induced brain tumours, and consequently supports the concept that ZIKV is safe and effective in a large model. Advertising the translation inherent safety issues with human translation, scientists have designed microRNA-reactive ZIKV variants (oZIKV) selectively silenced within normal tissue, to increase the safety of the application without impacting upon oncolytic activity (Novaes et al., 2024). Interestingly, such engineered viruses have been reported to pass through the BBB after systemic injection as well as to ameliorate survival rates in animals.

1.1 Evidence of Preclinical Applications in Experimental Models

There is a mature literature to support the oncolytic activity of Zika virus (ZIKV) against glioma. *In vitro* systems constantly reveal that ZIKV selectively infects and kills glioma stem cells (GSCs) without primarily impacting the rest of the differentiated neurons and astrocytes (Iannolo et al., 2019). The virus brings about the presence of apoptotic pathways, metabolic, and cytopathic effects in ZIKV-permissive tumour cells, contributing to its natural selection according to the progenitor-like populations.These are further supported by murine xenograft models. In the orthotopic model of experimental conditions, ZIKV delivered into tumours causes significant tumour regression, tumour reduction, and survival of the treated animals. Intraventricular and systemic modes of administration have shown effectiveness, and reports of using genetically engineered oncolytic ZIKV (oZIKV) have demonstrated that these viruses can cross the bloodbrain barrier (BBB), retention of tumour selectivity activity and ultimately induce considerable survival in the normal tissue without any neurotoxic effects.

The strongest translational support is that of a canine study where three dogs that supported malignant brain tumours spontaneously were intrathecally infected with ZIKV. It has been well tolerated with significant tumour shrinkage capacity as well as an improvement in neurological outcomes with no observed systemic viral disease manifestation thus showing both feasibility and initial safety in large immunocompetent hosts. These findings are supported by systematic reviews of ZIKV-based therapies in a variety of preclinical studies and accentuate the specificity of the oncolytic effect of ZIKV on the tumour of the central nervous system (de Sena Barbosa et al., 2024, Jiang et al., 2023).

1.2 Outcomes Translational considerations and blocking paths to clinical utilization

Despite promising data in the preclinical studies, the translational studies of ZIKV virotherapy into human trial requires careful evaluation of delivery strategies, biomarker identification, regulation issues, and combinatorial therapeutics possibilities (Calderón-Peláez et al., 2024).

Intratumoral or intraventricular delivery is the least toxic and the most restricted delivery route and ensures the greatest local concentration of virus, which reduces systemic distribution. However, there is still interest on systemic delivery in case of multifocal tumours or in tumours that are surgically inaccessible(Kaid et al., 2018; Kaid et al., 2022). OZIKV variants engineered to overcome the blood-brain barrier have shown this ability, so future systemic approaches can be an effective way to implement it in clinical practice.Since, the effect of innate immunity in GBM patients is diverse, predictive biomarkers, such as the breach of interferon signaling or unnatural expression of integrins that facilitate viral cellular invasion, can become valuable in stratifying the patients, thus supporting their efficacy and safety. The literature on more comprehensive oncolytic viruses points at the need of biomarker-based regimens of therapeutic approaches in glioma virotherapy.

A combination therapy is one of the promising directions in the future. The evasion of immune checkpoint inhibitors or radiotherapy could be reinforced by antigen release by the ZIKV and activation of the tumour microenvironment. Other oncolytic viruses have also been shown to have similar synergies and combining ZIKV with immunotherapeutic modalities may enhance its therapeutic effect (Asija et al., 2023).Control, as well as ethical issues, are also significant. The teratogenicity of ZIKV with its potential systemic spread, systemic dissemination, and the impact on the wellbeing of the population precondition high containment measures, as well as carefully developed exclusion criteria, especially those related to pregnant people and their close range of contacts. The policies will involve ethical review boards where extensive preclinical safety information and intensive justification of human trials will be demanded focusing on the necessity of complete safety testing.

1.3 Limitations of virotherapy

Inoculation ZIKV virotherapy is supported by sufficient evidence, most of which is still preclinical. Results obtained using *in vitro* assays, mouse xenografts, and a small group of dogs should be viewed with scepticism as these experimental models do not adequately reflect the complex nature of glioblastoma multiforme of humans (Francipane et al., 2021).

Inconsistencies in the experimental design, such as variations, among viral strains, models, experimental administering routes and the schedule of dosing create a huge challenge to standardization and roadblocks comparability across studies (Chen et al., 2019).

The endotoxin problems such as long-term toxicity, inflammatory residues as well as the viral persistence are understudied, and they have to be stringently evaluated prior to implementing clinical translation.

1.4 Clinical applications of virotherapy

Zika virus is a biologically engaging contribution to the current oncolytic changes in virotherapy. It has the unique therapeutic property of preferentially infecting neural progenitor-like populations endowing it with a unique therapeutic targeting ability to attack glioma stem cells- tumour cells associated with therapeutic resistance, recurrence and disease progression. The continued accrual of preclinical data in cellular models, murine xenografts and canine spontaneous-tumour models, continues to show that ZIKV can cause a robust tumour regression, neuro-attenuation and a re-organization of the tumour microenvironment to a more immunologically aroused state (Nizamutdinov et al., 2025).

However, the flow of the pre-clinical promise to the clinical application requires careful balance of safety, ethics, and biology issues. Wild-type ZIKV has been linked to neurodevelopmental toxicity and thus, careful engineering is necessary to reduce the risk. Innovations like microRNA-sensing strains of the ZIKV can represent a significant breakthrough since they offer tumour targeted replication without compromising oncolytic efficacy. Such innovations do provide a basis of clinical translation in the future and highlight the potential of ZIKV as an efficient adaptable therapeutic platform (Zhou et al., 2023).

The newly found information on ZIKV communication with glioma stem cells in addition to the immune landscapes changes and ways to traverse over the blood brain barrier barrier provides significant data not only to virotherapy studies but also to the area of neuro-oncology studies in general (Lang et al., 2018). In case of further research, which could prove to be safe, maximize engineered viral constructions and find the most efficient therapy regimens, ZIKV-based solutions could eventually become a breakthrough in modern glioblastoma treatment paradigms (Shoaf et al., 2022)

Conclusion

In conclusion, although there are still serious challenges, the current research directions are in high demand of the promise of ZIKV as a potential pathogen-attack force with the capability of being transformed into a safe, highly specific, and therapeutic tool against malignant glioma. Further multidisciplinary interactions, including virology, neuro-oncology, immunology, and genetic engineering, will define the timeline of further development of ZIKV virotherapy to first-in-humankind-studies as well as the provision of a new hope to patients diagnosed with one of the most extreme human cancers today.

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CHAPTER 4: The Tumour Microenvironment: An Emerging Frontier for Targeted Cancer Therapeutics

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Abstract

The tumour microenvironment (TME) is a complex, dynamic, and heterogeneous system that plays a crucial role in cancer development, progression, metastasis, and response to therapy. It consists not only of malignant cells but also various non-cancerous components such as cancer-associated fibroblasts (CAFs), tumour-associated macrophages (TAMs), endothelial cells, pericytes, and diverse immune cells, all embedded within an altered extracellular matrix (ECM). The interactions among these cellular and acellular components create a supportive niche that promotes tumour growth, immune evasion, and drug resistance.Tumours within the TME are characterized based on behaviour (benign, pre-malignant, malignant), cell type or origin (e.g., carcinoma, sarcoma, leukaemia), and histogenesis (ectodermal, mesodermal, endodermal). Additionally, tumours may be classified into special categories. Various carcinogenic agents contribute to tumour development, including chemical agents (e.g., N-nitrosamines, aromatic compounds), physical agents (e.g., ionizing and ultraviolet radiation), and biological agents such as viruses (e.g., papillomavirus, Hepatitis B and C), bacteria (e.g., Helicobacter pylori), and parasites.The primary aim of studying the TME is to understand how cancer cells interact with surrounding stromal, immune, and vascular elements to influence tumourbehaviour and treatment outcomes. This understanding aids in identifying diagnostic and prognostic biomarkers, uncovering mechanisms of drug resistance, and developing targeted therapies.Clinically, TME research has led to advances such as immunotherapy using checkpoint inhibitors and combination treatments targeting multiple components of the TME. Future research focuses on developing advanced models, discovering new therapeutic targets, and personalizing treatment strategies. Overall, targeting both cancer cells and their microenvironment offers promising avenues for improved and durable cancer therapies.

Keywords: Tumour Microenvironment (TME), Cancer Progression, Therapeutic Resistance, Cancer Initiation, Progression and Metastasis, Immune Evasion

1. Introduction

Cancer is no longer viewed as merely a disease of rogue cells growing in isolation. Over the past two decades, our understanding has fundamentally shifted to recognize tumours as complex, dynamic ecosystems- a revelation that has transformed the landscape of oncology research and therapeutic development. At the heart of this paradigm shift lies the tumour microenvironment (TME), a sophisticated cellular and molecular habitat that not only sustains malignant cells but actively promotes their survival, proliferation, and metastatic spread.The tumour microenvironment encompasses the entire non-cancerous cellular and acellular milieu surrounding tumour cells, including immune cells, fibroblasts, endothelial cells, pericytes, adipocytes, extracellular matrix (ECM) components, signalling molecules, and metabolic byproducts. Far from being passive bystanders, these elements engage in intricate bidirectional communication with cancer cells, creating a permissive niche that facilitates tumour progression while simultaneously suppressing anti-tumour immunity.

2. Types of tumours

Tumours are characterized on the basis of their (i) behaviour, (ii) origin/cell type, and (iii) histogenesis. Also, tumours are classified on the basis of some special categories.

2.1 On the Basis of Their Behaviour:

(i) Benign Tumours

These types of tumours, such as Lipoma and Fibroma, are not cancer-causing. They do not spread (metastasize) to the nearby tissues and other body parts (Figure 1). After removing them from the body, they generally do not return (Patel, 2020).

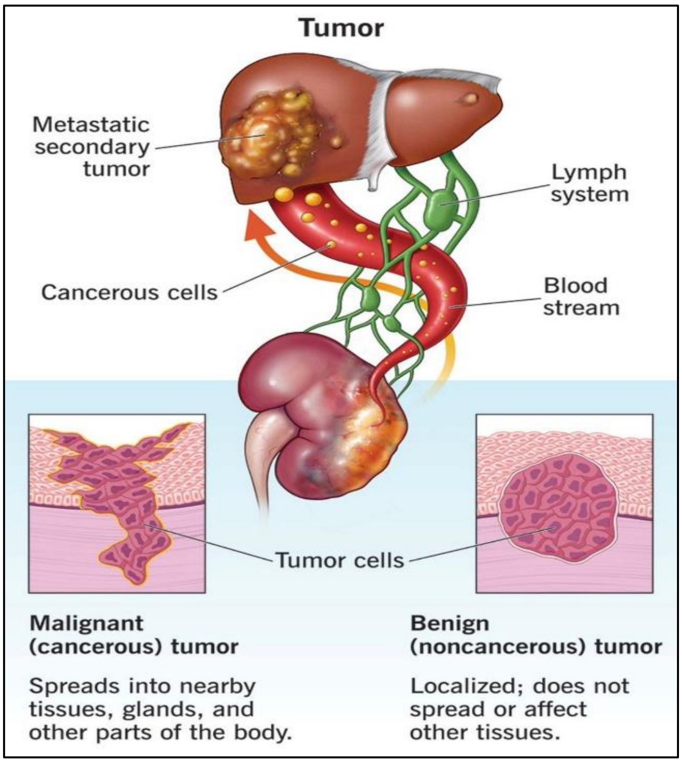

Figure1: An illustration of the complex cellular habitat surrounding a tumour, (adopted from Patel, 2020).

(ii) Premalignant Tumours

These types of tumours are not cancerous at the start, but they have full potential to become malignant.

(iii) Malignant Tumours

These types of tumours, such as carcinoma, sarcoma and leukemia, are cancer-causing. They spread to the nearby tissues and other body parts (metastasis)(Patel,2020).

2.2 On the Basis of Their Cell Origin or Histogenesis:

Tumour types	Origin	Example
Carcinoma	Origin of the tumours are on epithelial cells like lining of organs and skin etc	Breast, Lung, Prostate, Colon cancer
Sarcoma	These types of tumours occurred in the connective tissue like bone,cartilage, fat muscle etc	Osseosarcoma, Liposarcoma
Leukaemia	These types of tumours that are occurred in the blood forming tissues like bone marrow	Acute lymphoblastic Leukaemia,chronic myelogenous Leukaemia
Lymphoma	These types of tumours are observed in immune system cells like Lymphatic system	Hodgkin lymphoma, non-Hodgkin lymphoma
Myeloma	These are the plasma cell tumour	Multiple myeloma
Germ cell tumour	The origin of these tumours are pluripotent germ cells like testes and ovaries	Seminoma,Dysgerminoma
Blastoma	These tumours are originated in embryonic tissues	Neuroblastoma,Retinoblastoma
Others	Origin is nervous system	Glioma,Neningioma

2.3 On the Basis of Their Histogenesis

i. **Ectodermal**: These are the tumours on nervous system and also these are skin carcinomas.

ii. **Mesodermal**: These are sarcomas and leukaemias.

iii. **Endodermal**: These are gastrointestinal adenocarcinomas.

2.4 On the Basis of Their Special Types:

i. **Mixed Tumours**: Consist of more than one tissue type. Example: Pleomorphic adenoma of salivary gland.

ii. **Teratomas**: These are tumours that occur from germ cells containing hair, teeth, etc.

iii. **Blastomas**: These are tumours of precursor immature cells generally in childhood. Examples: Retinoblastomas, Neuroblastomas.

3. Tumour inducing agents

Tumour inducing agents are also generally called carcinogens. Carcinogens are all those substances or factors that can eventually give rise to a tumour by promoting abnormal cell growth. These agents are classified into some categories.

3.1 Chemical agents

Their molecules include chemicals that are used in industrial purposes like tobacco components, certain drugs, and some environmental pollutants. DNA mutations caused by chemical carcinogens that interrupt in cellular replication. Some examples are:

1. N-nitrosamines
2. Aromatic compounds like benzopyrene, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)
3. Certain metals and natural compounds like Aflatoxins (Luch,2005).

3.2 Physical Agents

DNA can be directly damaged by these types of physical agents that increase the rate of damage of other carcinogens (Luch, 2005). Example of physical agents are ionizing radiation, non-ionizing radiation and ultraviolet radiation.

3.2 Biological Agents

Some viruses (like Papillomavirus, Hepatitis B and C), parasites and bacteria (e.g. Helicobacter pylori) can cause chronic inflammation or induce their genetic material in the host cell system (Cummins and Tangney, 2013).

4 Tumour microenvironment

4.1 Growth Factors

Growth factors are very crucial regulators for the tumour microenvironment. This is evidenced in tumour initiation, progression and metastasis. The tumour microenvironment includes cancer cells and the surrounding stromal components (fibroblasts, endothelial cells, immune cells and extracellular matrix).Tumour microenvironment growth factors act in autocrine and paracrine signalling and are involved in cell proliferation and angiogenesis(Yuan et al.,2026) (Sabit et al.,2024). Growth factors like vascular endothelial growth factor (VEGF), epithelial growth factor, platelet-derived growth factors, transforming growth factor-β (TGF-β), and fibroblast growth factors are produced by both tumour and stromal cells. These factors stimulate cancer cell proliferation, enhancing their uncontrolled growth (Zhang et al., 2010; Yuan et al., 2016; Guo and Deng, 2018; Beyran et al., 2025).

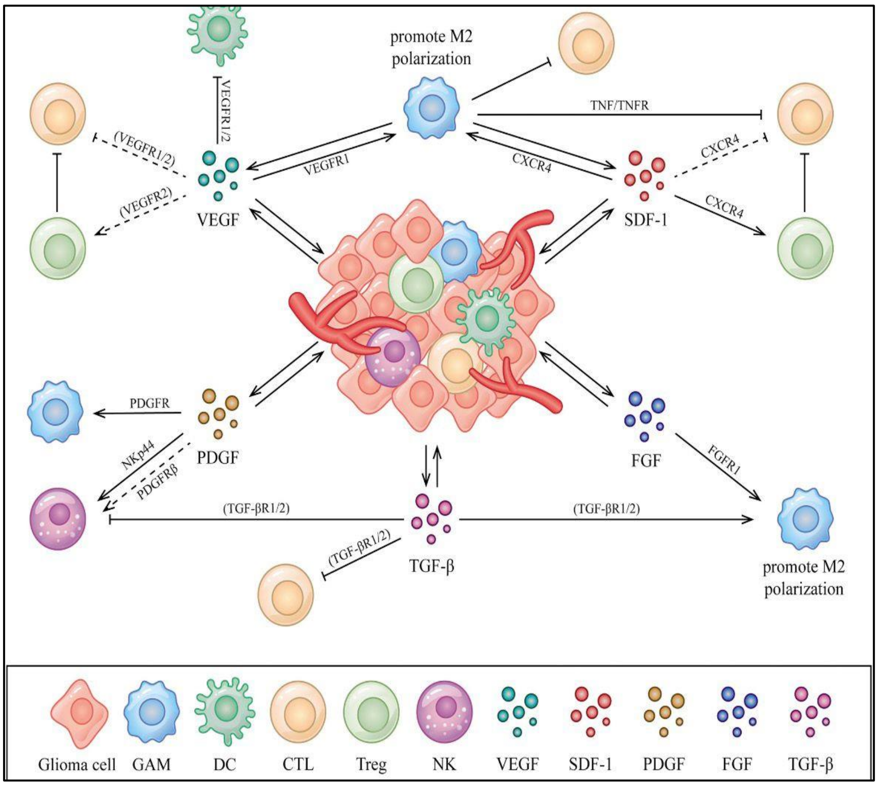

Figure 2: An overview of bidirectional signalling involving growth factors such as VEGF, PDGF, FGF, and TGF-β between glioma cells and various immune/stromal cells like GAMs, DCs, and CTLs (Yuan et al., 2016; Sabit et al., 2024).

4.2 Transcription Factors

Transcription factors are those regulatory proteins which can bind to a specific DNA sequence and control DNA transcription. Transcription factors have an essential role in shaping the tumour microenvironment by regulating gene expression in both surrounding stromal or immune cells and cancer cells. They have an impact on metastasis, growth, immune evasion and response to therapy (Table 1).

Table 1: Key Transcription factors in the tumour microenvironment

Transcription factors	Main role in TEM	Clinical impact
STAT3	Tumour progression, Immunosuppression	Targeted in trails for immunomodulation,
NF-kB	Cytokine production, inflammation and survival	Linked with cancer and chronic inflammation
HIF-α	Angiogenesis,Metabolism and Hypoxia response	Target for anti-angiogenic therapies
FoxP3	Regulatory T cell function	Target for reducing Terg-mediated suppression
Twist, Zeb, Snail	Metastasis and EMT	Linked to therapy-resistant tumours and metastatic

4.3 Pro-inflammatory Cytokines

Pro-inflammatory cytokines are kind of signalling proteins like Interleukin-6, Interleukin- 1 beta (IL-1β) and tumour necrosis factor α (TNF-α) - that plays an important role in tumour microenvironment (Table 2; Figure 3).

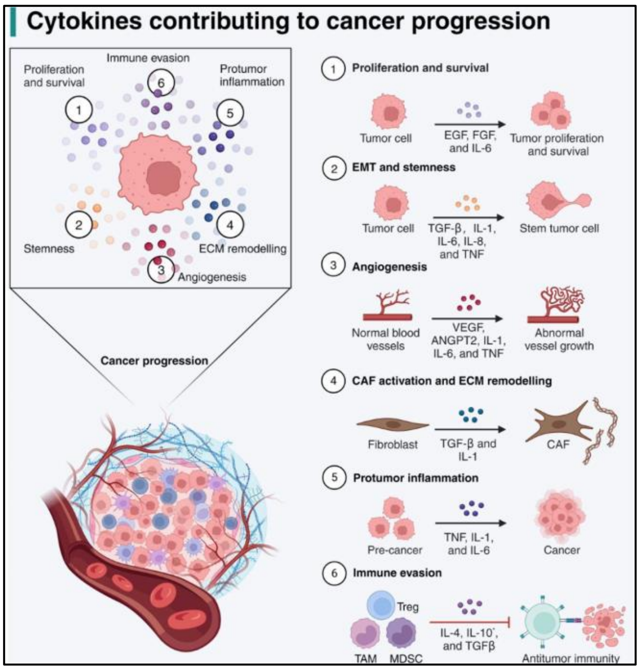

Figure 3: A multi-step visualization of how cytokines like IL-6, TNF, and IL-1 drive proliferation, stemness, angiogenesis, CAF activation, and immune evasion (adopted from Landskron et al., 2014).

Table 2: Role of different pro-inflammatory cytokines (adapted from Landskron et al., 2014)

Cytokines	Mechanisms	Effect on tumour	Impact on immune system	Clinical significance
IL-6	Activates JKA/STAT3 pathway	Promotes proliferation, Metastasis, Angiogenesis	Suppresses Cytotoxic –T cells and recruit immunosuppressive cells	Target for cancer therapy trials
TNF-α	Activates NF-kB pathway	Stimulate invasion and tumour growth	Promotes immune-suppression and recruits inflammatory cells	Targeted inhibitors under study
IL- 1β	Induces inflammatory signalling	Enhances survival and progression of tumour cells	Attracts immune cells and supports Chronic inflammation	Potential therapeutic target

5 Tumour and malignancy two vis-a-vis events

When we put tumour and malignancy side by side (vis-a-vis), we see that a tumour is a broader term that includes both harmless (benign) and harmful (malignant) growths, whereas malignancy is the dangerous subset of tumours characterized by invasion, metastasis, and high risk to life. In other words, all malignancies are tumours, but not all tumours are malignant. A tumour represents the physical manifestation of cancer- the tangible, measurable mass of abnormal tissue that can be seen on scans, felt during examinations, and surgically removed. It has defined boundaries, occupies space within the body, and creates mechanical effects through its presence.

Whether appearing as a small nodule in the lung or a large mass in the abdomen, the tumour is cancer's visible face, the concrete evidence that something has gone wrong with normal cellular growth patterns. Malignancy, in contrast, describes the invisible biological behaviour that drives cancer's destructive potential. It encompasses the cellular processes that allow cancer cells to invade healthy tissues, resist normal death signals, and spread throughout the body. Malignancy cannot be surgically excised because it is not a thing but rather a set of acquired cellular capabilities-the biological blueprint that transforms ordinary cells into agents of disease. Every malignancy is a tumour, but not every tumour becomes malignant. Their interplay reflects the continuum from abnormal but localized growth to invasive, metastatic disease that threatens life.

6 Therapeutic approaches adopted so far for TME:

Therapeutic approaches adopted so far for the tumour microenvironment refer to several strategies that target the non-cancerous supporting components of a tumour such as stromal cells, immune cells, signalling molecules, and extracellular matrix. Tumour microenvironment includes stromal fibroblasts, immune cells, blood, lymphatic vessels, and cellular extracellular matrix, which encourage cancer progression. Tumour cells co-opt natural cells to release growth factors like cytokines and other enzymes that promote proliferation and invasion. Stromal changes (e.g., high fluid pressure, altered blood flow) make drug delivery less effective. Tumour cell and TME cells are inhibited by multi-targeted approaches that may improve therapy. The therapeutic approaches have progressed from radiation, surgery, chemotherapy to targeted therapies and immunotherapy.

6.1 Conventional Approaches

- Surgery: Removal of localized tumour masses.
- Radiotherapy: Uses ionizing radiation to kill rapidly dividing cells.

- Chemotherapy: Cytotoxic drugs targeting proliferating cells (e.g., alkylating agents, antimetabolites, topoisomerase inhibitors).

6.2 Targeted & Modern Therapies

- Hormonal therapy: For hormone-dependent cancers (e.g., tamoxifen for breast, flutamide for prostate).
- Targeted therapy: Drugs that inhibit specific molecules driving cancer (e.g., Imatinib for BCR-ABL in CML, EGF inhibitors).
- Monoclonal antibodies: Rituximab (CD20), Trastuzumab (HER2).

6.3 Immunotherapy-Based Approaches

CAR-T Cell Therapy Modifications: Chimeric antigen receptor (CAR) T-cell therapy has shown remarkable success in haematological malignancies, but faces challenges in solid tumours due to barriers in the tumour microenvironment, including metabolic competition, physical barriers to infiltration, and immunosuppressive factors. Current strategies include structurally altering CAR-T cells combined with targeted therapy, radiotherapy, or chemotherapy to enhance infiltration and overcome immunosuppressive responses. Reshaping the tumour immune microenvironment aims to improve CAR-T-cell-based cancer immunotherapy.

6.4 Emerging Therapies:

- Gene therapy & CRISPR-based editing: Correcting oncogenes or tumour suppressor mutations.
- Oncolytic viruses: Viruses engineered to selectively kill tumour cells.
- Nanomedicine: Nanoparticles delivering drugs specifically to tumour tissue(Joyce,2005).

7 Conclusion and future prospective

Knowing the tumour microenvironment is very important for developing the cancer therapies. The TME (Tumour Microenvironment) not only favours tumour development and progression but also determines the success or failure of anticancer strategies. By manipulating or studying the TME, it is possible to design new treatment and to strengthen the therapeutic processes. It will help in better clinical outcomes for cancer patients.

The future prospective of the study of TME will be expanded by manipulation efforts to unveil and meaning of human microenvironment with more accuracy, enhance the treatment for cancer patients with more knowledge about TME by development of new immune modulatory agents, combination therapies etc.

Declaration of competing interest

The authors declare that they have no known financial or personal conflicts of interest that could have influenced the work presented in this paper.

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CHAPTER 5: Deep Brain Stimulation for Treatment-Resistant Depression

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Abstract: Treatment-resistant depression (TRD) affects approximately 30% of patients with major depressive disorder and represents a significant clinical challenge with limited therapeutic options. Deep brain stimulation (DBS), a neurosurgical intervention involving electrical stimulation of specific brain regions, has emerged as a promising treatment modality for severe, refractory depression. This review examines the neurobiological rationale, target regions, clinical efficacy, safety profile, and mechanisms of action of DBS in TRD. Analysis of clinical trials reveals variable response rates ranging from 20% to 60% depending on target site, patient selection, and stimulation parameters. The subcallosal cingulate cortex, ventral capsule/ventral striatum, and medial forebrain bundle represent the most extensively studied targets, each demonstrating distinct therapeutic profiles and side effect patterns. While DBS shows promise for carefully selected patients who have exhausted conventional treatments, challenges remain regarding optimal target selection, programming protocols, patient stratification, and long-term efficacy. Advances in neuroimaging, closed-loop systems, and personalized medicine approaches may enhance treatment outcomes and identify patients most likely to benefit from this invasive intervention.

Keywords: Deep brain stimulation, Treatment-resistant depression, Neuromodulation, Subcallosal cingulate, Major depressive disorder

1. Introduction

Major depressive disorder (MDD) affects over 300 million people worldwide and represents a leading cause of disability globally (World Health Organization, 2017). While pharmacotherapy and psychotherapy provide relief for many patients, approximately 30% develop treatment-resistant depression (TRD), defined as inadequate response to at least two adequate trials of antidepressant medications from different pharmacological classes (Gaynes et al., 2020). These patients experience persistent symptoms, functional impairment, and elevated suicide risk despite conventional interventions (Fava, 2003).

Deep brain stimulation (DBS), initially developed for movement disorders such as Parkinson's disease, has emerged as an investigational treatment for severe, refractory psychiatric conditions including TRD (Mayberg et al., 2005). DBS involves surgical implantation of electrodes into specific brain regions, delivering continuous electrical pulses that modulate neuronal activity within targeted circuits (Lozano & Lipsman, 2013). Unlike ablative neurosurgical procedures, DBS is reversible and adjustable, allowing optimization of stimulation parameters to maximize therapeutic benefit while minimizing adverse effects (Holtzheimer & Mayberg, 2011).

The neurobiological rationale for DBS in depression stems from neuroimaging studies demonstrating dysregulation of mood-regulating circuits involving the prefrontal cortex, anterior cingulate cortex, striatum, thalamus, and limbic regions (Drevets et al., 2008). Hyperactivity in the subcallosal cingulate cortex (SCC) has been consistently observed in depressed patients, and modulation of this region through DBS has shown clinical promise (Mayberg et al., 2005). Alternative targets including the ventral capsule/ventral striatum (VC/VS), nucleus accumbens (NAcc), medial forebrain bundle (MFB), and lateral habenula have also been investigated (Bewernick et al., 2010; Schlaepfer et al., 2013).

2. Deep Brain Stimulation: Technology and Mechanisms

2.1 DBS System Components

A typical DBS system consists of three components: the electrode lead, the extension wire, and the implantable pulse generator (IPG) (Lozano & Lipsman, 2013). The electrode lead, typically containing four platinum-iridium contacts, is stereotactically implanted into the target brain region under image guidance using magnetic resonance imaging (MRI) or computed tomography (CT) (Goodman & Alterman, 2012). The extension wire connects the electrode to the IPG, which is implanted subcutaneously in the chest wall, similar to a cardiac pacemaker (Holtzheimer & Mayberg, 2011).

Modern DBS systems allow programming of multiple parameters including voltage or current amplitude, pulse width, frequency, and contact configuration (Volkmann et al., 2006). Stimulation frequencies typically range from 90 to 185 Hz, though lower frequencies (20-50 Hz) have been explored for specific applications (Bewernick et al., 2010). Pulse widths generally vary between 60 and 450 microseconds, while amplitudes range from 1 to 10 volts or 1 to 25 milliamperes depending on the system type (Lozano & Lipsman, 2013).

2.2 Mechanisms of Action

The precise mechanisms by which DBS alleviates depression remain incompletely understood, though multiple effects on neural circuits have been identified (McIntyre et al., 2004). Contrary to initial assumptions that DBS inhibits neuronal activity, evidence suggests complex modulation involving both excitation and inhibition depending on stimulation parameters and neuronal elements affected (Lozano & Lipsman, 2013).

DBS influences local neurons, axonal projections, and synaptic transmission within stimulated regions and downstream targets (Hamani & Temel, 2012). Electrophysiological studies demonstrate that DBS can suppress pathological oscillatory activity, enhance information processing, and restore functional connectivity within disrupted mood-regulating networks (Horn et al., 2017). Neurochemical changes accompany DBS-induced circuit modulation, including alterations in serotonergic, dopaminergic, and glutamatergic neurotransmission (Hamani & Nobrega, 2012).

3. Clinical Efficacy by Target Region

3.1 Subcallosal Cingulate Cortex (SCC)

The subcallosal cingulate cortex, also known as Brodmann area 25, represents the most extensively studied DBS target for TRD (Mayberg et al., 2005). The open-label study by Mayberg et al. (2005) reported 60% response rates (>=50% reduction in Hamilton Depression Rating Scale scores) in six patients at 6-month follow-up. However, the pivotal BROADEN trial, a multicenter randomized sham-controlled study, failed to meet its primary endpoint at 6 months, showing no significant difference between active and sham stimulation (Holtzheimer et al., 2017).

3.2 Ventral Capsule/Ventral Striatum (VC/VS)

The ventral capsule/ventral striatum target encompasses white matter tracts connecting prefrontal cortex with subcortical structures, including the nucleus accumbens (Malone et al., 2009). The RECLAIM trial, a randomized sham-controlled study in 30 patients, demonstrated a 44% txt response rate with active stimulation compared to 20% with sham at 16 weeks, though this difference did not reach statistical significance (Dougherty et al., 2015).

Table 2.1: Clinical Outcomes of DBS for Treatment-Resistant Depression by Target Region

Target Region	Study Design	Sample Size	Response Rate (%)	Reference
SCC (Cg25)	Open-label	20	60%	Mayberg et al. (2005)
SCC (Cg25)	Open-label	21	62%	Kennedy et al. (2011)
SCC (Cg25)	RCT	90	28% (vs 17% sham)	Holtzheimer et al. (2017)
SCC (Cg25)	Conn.-guided	11	82%	Riva-Posse et al. (2018)
VC/VS	Open-label	15	53%	Malone et al. (2009)
VC/VS	RCT	30	44% (vs 20% sham)	Dougherty et al. (2015)
VC/VS	Ext. Follow-up	30	70%	Dougherty et al. (2015)
MFB	Open-label	7	71%	Schlaepfer et al. (2013)
MFB	Open-label	16	50%	Bewernick et al. (2017)
NAcc	Open-label	10	50%	Bewernick et al. (2010)

4. Safety and Adverse Effects

DBS implantation carries inherent surgical risks associated with cranial neurosurgery. Intracranial hemorrhage occurs in approximately 1-2% of procedures and can result in permanent neurological deficits. Infection rates range from 3-8% and may necessitate hardware removal (Fenoy & Simpson, 2014). Adverse effects directly attributable to electrical stimulation are typically reversible through parameter adjustments and include transient anxiety, hypomania, and paresthesias (Malone et al., 2009).

Table 2.2: Adverse Events Associated with DBS for TRD

Category	Adverse Event	Frequency	Management
Surgical	Intracranial hemorrhage	1-2%	Surgical evacuation if clinically required
Surgical	Infection	3-8%	Antibiotic regimens, transient hardware removal
Surgical	Lead misplacement	1-5%	Stereotactic surgical revision
Hardware	Device malfunction	5-10%	Surgical component replacement
Stimulation	Anxiety / agitation	5-15%	Clinical parameter adjustment
Stimulation	Hypomania / mania	2-8%	Parameter down-titration, pharmacology
Stimulation	Cognitive alterations	5-10%	Parameter re-optimization
Stimulation	Paresthesias	3-7%	Contact modification and shielding
Psychiatric	Suicidal ideation	Variable	Intensive psychiatric intervention & protection

5. Challenges

Optimal patient selection remains a critical challenge, as not all TRD patients benefit uniformly from DBS. Current selection criteria include failure of multiple adequate antidepressant trials, absence of active substance abuse, and absence of severe personality pathology (Holtzheimer & Mayberg, 2011). Current open-loop setups are progressively transitioning toward closed-loop adaptive systems that sense neural oscillatory patterns and autonomously modulate current delivery on-demand (Widge et al., 2018).

6. Conclusion

Deep brain stimulation represents a promising intervention for carefully selected patients with severe, treatment-resistant depression who have exhausted conventional therapeutic options. Clinical trials across multiple target regions demonstrate variable but meaningful response rates, with some patients achieving complete remission. The subcallosal cingulate cortex, ventral capsule/ventral striatum, and medial forebrain bundle have emerged as the most studied targets, each offering distinct therapeutic profiles. Future research focused on tracking electrophysiological biomarkers and adaptive closed-loop paradigms will continue to refine outcomes for refractory mental health disorders.

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CHAPTER 6: Nano-Biopesticides: Review on an Elegant Contribution of Nanotechnology on Environment

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Abstract

Nanotechnology and biotechnology are two quickly developing technologies that, when combined, provide promising agricultural solutions in the form of nano-biopesticides. Conventional pesticides have several drawbacks, including low efficacy, high toxicity, poor dispersibility, and environmental contamination. Made from biological sources such as microorganisms and plants, these offer safer substitutes because of their low toxicity, quick biodegradability, and target specificity. Effective and environmentally friendly nano-biopesticides are produced by incorporating nanotechnology into biopesticide formulations to improve stability, solubility, bioavailability, and controlled release. A variety of nano-bioformulation techniques, including nanoencapsulation and nano-emulsions,which shield active chemicals from deterioration, allow for continuous release, and enhance absorption, can be used to create nano-biopesticides. These innovative formulations have shown promise in controlling nematodes, fungal infections, and insect pests while causing the least amount of damage to organisms that are not the intended target. Beyond pest management, applications include precision farming, crop protection, and food preservation using antimicrobial systems based on nano-emulsions. Current viewpoints emphasize how nanoparticles can both decrease pests and boost plant development by improving photosynthetic efficiency and environmentally and human-safe nano-biopesticides.Although nano-biopesticides offer viable substitutes for chemical pesticides and have the ability to guarantee food security, a thorough assessment of their long-term effects on the environment and human health is still necessary.

Keywords: Crop protection, Food security, Nano-biopesticides, Nano-bioformulation,Nano-emulsion, Pest Management

1. Introduction

Biotechnology and nanotechnology are two of the most promising technologies of the twenty-first century. Designing, creating, and using materials and devices with the least amount of functionality occurring on a nanoscale scale (1 to 100 nm) is known as nanotechnology. The metabolic and other physiological changes of biological beings, including microbes, are the focus of biotechnology. It is implied that these two technologies (i.e., nanobiotechnology) can be crucial in creating and utilizing numerous useful instruments in the study of life. These microbial processes have created new avenues for investigating innovative applications, such as the biosynthesis of metal nanomaterials.From extensions of traditional device physics to entirely new strategies based on molecular self-assembly, from creating new materials with nanoscale dimensions to examining whether we can directly control matters on/in the atomic scale level, nanotechnology is incredibly diverse. This concept involves applying it to a variety of scientific domains, including organic chemistry, plant biology, agriculture, the food industry, and more(Lade et al.,2019).

The high organic solvent content, dust drift, poor dispersibility, long half-life in the soil, and other related issues are some of the limitations of conventional pesticide formulations. Only 1% of pesticides remain on surfaces as a result of these restrictions; the majority are discharged into the atmosphere. The environment is severely contaminated as a result of this inefficiency. Therefore, it is important to reduce pesticide waste, manufacturing expenses, and environmental emissions in addition to prolonging the time that pesticides are effective on crops. To avoid this problem some researchers studying pest control are focusing on developing non-toxic substitutes for synthetic chemicals used to control diseases and pests. The application of biopesticides is one of these substitute techniques. Biopesticides, sometimes referred to as "biological pesticides," are used in place of conventional pesticides to manage plant pests. The main benefits of using natural substances as biopesticides as opposed to conventional pesticides are as follows: (i) reduced toxicity; (ii) exclusive to the offending pest and closely related species; (iii) strong potency at low dosages; (iv) quick breakdown; and (v) minimal exposure with virtually no emission problems (Abdollahdokht et al., 2022)

Nanobiopesticides is one of the biopesticides that is invented through nanotechnology.

Comprehending the interactions between nanomaterials and biopesticides is crucial for enhancing their synergistic benefits in pest management and reducing environmental hazards. Microbial nano-biopesticides employ microorganisms as the active component, integrating nanomaterials to enhance stability and effectiveness.

In short, a major development in pest management techniques is the switch from conventional pesticides to nano-biopesticides. Sustainable alternatives must be used in place of conventional pesticides due to their negative environmental effects. The technology known as nano-formulation of pesticides was created and plays a significant part in plant conservation. By utilizing nanomaterials and biopesticides in a novel way, nano-biopesticides present a promising approach that minimizes environmental impact while offering effective and targeted pest control. Optimizing the effectiveness of nano-biopesticides and making sure they are used responsibly in agriculture require an understanding of their categorization and processes. Adopting these ecologically friendly methods will be crucial to maintaining the health of our ecosystems and guaranteeing food security as the agriculture industry develops further (Anjaneyulu et al.,2024).

2. Biopesticides & its function

The term "biopesticides" describes specific pesticides that can originate from any biological form, including bacteria, plants, animals, etc. Their non-toxic mode of action makes them a promising substitute for environmentally friendly insect pest management. The pathogenicity of a microbe to a target pest organism informs the design of microbial bio-pesticides. It has been shown that the antibiotic metabolites of certain bacterial species from the genera Bacillus and Pseudomonas are efficient bio-control agents. The United States, Australia, Russia, and others are advancing the development of novel fungicides. Only a small number of entomic infections, meanwhile, have been created as biocontrol agents. One possible option that functions as a safe microbial pesticide is Trichoderma species. These days, China, Russia, Belarus, and to a lesser degree, India and Thailand, are essential producers of Bacillus thuringiensis products that are widely utilized. Bt. delta-endotoxin Cry1C has been shown in numerous investigations to have insecticidal effect, demonstrating the potency of bacterial biopesticides.The component of biopesticides acting as a biopesticide that can be used as a crop protection product and needs to be registered. Microorganisms are stimulated by plants through the process of sap ascension. Additionally, understanding the many ways in which the biopesticide acts during fungal-host interaction is crucial. Numerous microbes compete with this process.The biopesticide's effect is a combination of its several ways of action. For instance, Trichodermaharzianum competes with antibiosis and causes resistance in plants as a result. It is evident from the January 2015 situation that the majority of tomato and sweet pepper growers employed integrated control for the majority of plant diseases and insect pests, virtually all year long. One could argue that eight novel biopesticides were tested in order to facilitate registration under a "Green Deal process."

4. Nano-biopesticides

In order to increase the effectiveness, solubility, target-specific delivery, and controlled release of active ingredients, biologically derived pesticides are mixed with nanoparticles to create nanobiopesticides. To improve spreadability, stability, and biodegradability while preserving low residue levels in plants, they can be made using polymers, metal oxides, micelles, or biocomposites. Examples include pyrethrin-based nanoparticles that are harmful to Spodoptera littoralis and silver nanoparticles derived from extracts of Ficus species that are intended to target insect pests. Although they are effective even at low dosages, questions about their disposal and environmental safety still need to be answered, emphasizing the importance of thorough testing before usage.The limitations of traditional chemical pesticides that affect people, animals, and beneficial insects are addressed by nanotechnology, which provides environmentally acceptable alternatives for agricultural pest management. By delivering precise doses to plant tissues, nano-biofungicides formulations utilizing organic and inorganic nanomaterials such silica nanoparticles, titanium dioxide, nanoemulsions, and nanoclays reduce waste and their negative effects on the environment. They can successfully fight off fungal infections such as Botrytis cinerea, Fusarium spp., and Rhizoctonia solani. One prominent example is "Diyarex Gold," a certified residue-free bio-fungicide created by R.V. Agri Corporation, that is safe for both beneficial insects and people and effective against rust, blight, and powdery mildew. Nanoparticles of sizes between 10 and 1,000 nm or less than 100 nm are used in nanotechnology. The majority of metallic nanoparticles are made from metals like copper, platinum, silver, and gold. One new area of study in contemporary science is nanotechnology. The life sciences, particularly biotechnology and biomedical devices, are evolving daily. Numerous industries, including chemical, agricultural, medicinal, biological, and cosmetics, can benefit from nanotechnology. Mesoporous silica nanoparticles were initially employed as a water-soluble insecticide to combat validamycin. Sitophilus oryzae L. and Rhyzoperthadominica, two significant insect pests in food storage, were combated with alumina nanoparticles. Food packaging, textiles, disinfectants, and a number of home appliances all contain silver nanoparticles.

5. Nano-bioformulation

Basically this is a technique through which Nanobiopesticides are formed, combining the two processes nanoformulation (increasing properties like stability, solubility, bioavailability etc.) & bioformulation (synthesizing microbes) together.

Formulations of bio-based nanoparticles (NPs), especially silver NPs made at room temperature with plant extracts or microbes, provide an economical, recyclable, and energy-efficient solution. Stable NPs with exact size, shape, and composition can be created by refining reaction conditions and organism selection. Helicoverpa armigera and other pests have been successfully managed with these. By encasing the bioagent in a polymer (protein or carbohydrate) that crosslinks with the biopesticide-based NP, controlled release is accomplished, allowing for targeted and extended pesticidal action.With problems including toxic solvents, poor dispersion, and dust drift, more than 90% of conventional pesticides leak into the environment and leave behind hazardous residues in crops. These issues are resolved by nano-based pesticide formulations, which allow for the exact, trigger-responsive release of active chemicals. Creating water-based dispersions, boosting bioavailability, strengthening leaf-targeted delivery and dose transfer, and guaranteeing natural breakdown with safe residues are important tactics(Lade et al.,2019).

Types of Nano-bioformulation

5.1. Nano-encapsulation : Nano-encapsulation is a pesticide delivery method where active ingredients (AIs) are enclosed within organic (e.g., polymers, lipids, plant-derived nanoparticles) or inorganic (e.g., silica, carbon, calcium, clay) nanomaterials for controlled release. This approach protects AIs from degradation, reduces losses, improves uptake and bioavailability, and enables sustained or stimuli-responsive release triggered by environmental or biological factors. Polymer-based systems (nano-capsules, spheres, gels, fibers) offer biocompatibility and versatility, while lipid-based systems enhance penetration without harmful solvents. Plant virus nanoparticles have been used to improve AI mobility in soil. Inorganic porous materials provide high loading, tunable pore size, and enhanced stability, with tailored structures to control release and adhesion.Polymer instability and AI degradation due to environmental stressors are obstacles despite the advantages. AIs that are hydrophilic or hydrophobic can be delivered by vesicle-based carriers (liposomes, niosomes), with modifications such as chitosan coating or photo-responsive systems to increase durability, target delivery, and decrease toxicity. (Abdollahdokht et al.,2022)

5.2. Nano-emulsions : Oil-in-water dispersions known as nano-emulsions, which have droplets as small as 20 to 200 nm, increase the solubility, bioavailability, and effectiveness of pesticides that are not very soluble in water while using fewer organic solvents and surfactants. They provide superior wetting, spreading, penetration, and leaf coverage over conventional formulations. Examples include nano-emulsions of abamectin and glyphosate, which have reduced toxicity, more biological activity, and smaller droplet sizes than commercial versions. Even though high-energy emulsification is frequently used, hazardous substances may be present. Green nano-emulsions, which use eco-friendly surfactants and renewable oils, including β-cypermethrin systems with high stability (PDI < 0.2), offer a more biocompatible and sustainable method of delivering pesticides (Abdollahdokht et al.,2022).

6. Application of Nano-biopesticides

6.1. In Agriculture: More than 70% of important crop diseases are caused by fungus pathogens, and the traditional agrochemicals used to manage them are expensive, hazardous, and becoming less effective as a result of pest resistance. By employing nanoparticles (NPs) for targeted disease management, agronanotechnology provides a sustainable substitute. Several bacterial and fungal diseases can be effectively combated by silver nanoparticles (AgNPs); at low concentrations (as low as 3 ppm), nanosilver (1-5 nm) exhibits superior fungal suppression. Other NPs with antifungal and antibacterial qualities include copper, zinc, titanium, magnesium, gold, alginate, and silica; some of them have lower plant toxicity than AgNPs. Pesticide use can be decreased and resistance can be postponed by combining NPs with biocontrol agents, essential oils, or bioorganic pesticides. Smart NP-based delivery methods, such as photocatalytic "green nanocides" and nano-dispersed fungicides, increase efficiency, lower toxicity, and are appropriate for low-cost, large-scale manufacturing. Furthermore, NP-assisted plant breeding, genetic engineering, and nano-diagnostics can create crops that are resistant to disease, improving sustainable agriculture and long-term plant protection.In addition to abiotic (storms, droughts, floods) and biotic (particularly insect pests), which result in the largest agricultural losses roughly 14% each year, valued at $2 trillion population increase is putting strain on global agriculture. Traditional farming practices including crop rotation, improved cultivars, rescheduled planting dates, and integrated pest management (IPM) are losing their effectiveness. Chemical pesticides cause additional issues such limited effectiveness, pest resistance, and environmental damage. In order to better protect crops while using fewer pesticides, new, host-specific, targeted pest management techniques are required. Nematodes are extremely damaging soil pests that, in tropical climates, cause an estimated $125 billion in agricultural losses annually. They do this by feeding on roots, which lowers plant health, production, and nutrient intake while also increasing susceptibility to secondary illnesses. Due to the lengthy breeding process, there aren't many crop kinds that are resistant to nematodes. With pesticides offering severe toxicity hazards, traditional management techniques like crop rotation, trap crops, and chemical nematodes provide little or no protection. Silver, ZnO, CeO_2, TiO_2, and Al_2O_3 nanoparticles (NPs) are efficient against a variety of nematode species, including Meloidogyne spp. and *Caenorhabditis elegans*. Abamectin encapsulated in virus-based NPs greatly reduced illness in tomatoes. It has also been demonstrated that magnetic FeO_4 NPs cause apoptosis, change gene expression, and hinder worm motility(Yadav et al.,2018).

6.2. In Crops protection :

Implementing cutting-edge technologies like nanotechnology can significantly improve agriculture in underdeveloped countries by enabling "precision farming," smarter farming, and sustainability. Plant diseases reduce crop productivity by 10–20%, necessitating effective disease management. Precision farming reduces waste and pollution by integrating nanosensors, wireless networks, GPS, and remote sensing to monitor environmental conditions and offer focused responses, including automated pesticide application. Delivery technologies based on nanoparticles (NP), such as nanocapsules and nanoemulsions, improve pesticide stability, decrease toxicity, allow for gradual release, and cut expenses by protecting specific crops. New developments in agronanotechnology, such as hybrid nanostructures, gene transfer, and intelligent pesticide delivery, have the potential to lower agriculture's carbon footprint and increase sustainability over the long run(Yadav et al.,2018).

Comparing nanofertilizers and nanoencapsulated herbicides to traditional agrochemicals, the former offer lower environmental impact, increased yields, and greater nutrient usage efficiency. Up to 60 days of extended nitrogen availability are offered by slow-release urea nanoformulations containing montmorillonite, nanozeolite, or hydroxyapatite. This increases grain N content and yields while lowering leaching. Phosphate-induced water eutrophication is reduced while soybean growth and biomass are increased by apatite nanoparticles. In crops like saffron, cucumber, and wheat, foliar sprays of Fe, P, and K nanofertilizers have greatly improved yield and quality characteristics while correcting micronutrient deficits. When herbicides like ametryn, atrazine, and simazine are nanoencapsulated in solid lipid nanoparticles or biodegradable nanocapsules, the toxicity to non-target organisms is decreased, stability is increased, controlled release is guaranteed, and high association efficiency (>84%) is maintained(Pandey et al.,2016).

6.3. Prohibition of food ruination:

Essential oils with antibacterial, antifungal, and antiviral qualities, like carvacrol, eugenol, citral, thymol, and vanillin, are useful natural preservatives in the food sector. These oils, which frequently contain substances like pinene, limonene, menthol, and benzaldehyde, demonstrate potent antibacterial activity against a variety of food-borne bacteria, viruses, fungus, and spores when added to nanoemulsions. Preservation techniques based on nanoemulsions are becoming more popular as substitutes for managing food product infections. Furthermore, essential oils can be made more effective by mixing them with natural antimicrobials like nisin, particularly when D-limonene is present(Hashim et al.,2018).

7. **Current perspective**

Many plants, including neem (Azarachta indica) extracts, Acorus calamus, Annona squamosa, Vitex negundo, Gnidia glauca, Toddalia asiatica, Argimonemaxicana, and Calotropis procera, have been shown to have pesticidal properties when made into stable metallic nanoparticles. Additionally, nanoparticles are employed to evaluate the physiology and growth of plants. Applications of nano-TiO2 have been shown to triple the photosynthetic rate and boost the chlorophyll content by 45%. The outcome was a 73% increase in dry weight. Furthermore, it has been noted that the germination efficiency increases with the size of the nanoparticle.Thenanosize of TiO2 may also speed up the breakdown of organic materials, improve the absorption of inorganic nutrients, and quench oxygen free radicals produced during photosynthetic activity, all of which could boost the rate of photosynthetic activity. Another benefit of nanoparticles is that there has been no evidence of any harm to soil microorganisms in a number of instances(Lade et al.,2019).

8. Future perspective

Future application strategies will generally focus on developing nanobiopesticides that are quickly biodegradable, have minimal phytotoxicity, have no negative effects on seed germination, and, most importantly, do not negatively affect human health in order to meet institutional standards for nanoregularity. The products' phototoxicity can be considerably decreased, according to the findings. Using a nanocoating made of biocompatible polyvinylpyrrole, for instance, reduced the phototoxicity of the powdered Ag nanoparticles. In addition to increasing the likelihood of using nanoparticles for improved plant germination and growth, the biocompatible coverings reversed the toxicity of the nanomaterials. Several nanopesticide delivery methods have been lately suggested for plant protection, including nanoemulsions, nanoencapsulates, nanocontainers, and nanocages and they have a great probability in future time.

According to recent research, nanobiopesticides can provide target-specific crop pest control while lessening the negative effects of chemical pesticides. They can aid in the creation of intelligent nanosystems that minimize negative issues for agriculture, including food security, productivity, and environmental imbalance. The controlled-release patterns of active substances that these nanosystems have demonstrated make them more effective for extended use; they can also address the issues of eutrophication and the buildup of residual pesticides. Numerous evaluations have been published to demonstrate the potential of biopesticides based on nanotechnology. It is yet unknown what will happen to plants, people, and animals in the end. The formulation, characterization, shape, and use of nano-biopesticides require a rigorous research approach(Lade et al.,2019).

9. Conclusion

Biotechnology and nanotechnology integration has created revolutionary opportunities in agriculture, especially in the area of pest control. Even though conventional pesticides work well at first, their low dispersibility, high toxicity, extended persistence, and massive environmental waste provide serious ecological and health risks. These flaws jeopardize human health and biodiversity while also diminishing agricultural viability over the long run. Therefore, the creation of targeted, effective, and environmentally benign substitutes like - biopesticides and subsequent developments into nano-biopesticides - is a crucial step forward. Biopesticides made from natural biological agents like fungi, bacteria, and plants provide a number of benefits, including low toxicity, pest-specificity,low residues, and quick breakdown. Their stability, shelf life, and effectiveness in outdoor settings are frequently restricted, though. Here’s where nanotechnology comes in very handy. Through the integration of nanoparticles into biopesticide formulations, scientists have effectively improved the active ingredients’ solubility, stability, bioavailability, and controlled release characteristics, resulting in the development of sustainable and potent nanobiopesticides. With the use of several nanoformulation processes, including nanoencapsulation and nanoemulsions, which protect active ingredients from premature degradation and enable stimuli-responsive release, nano-biopesticides can be created. This compositions eliminate the need for frequent treatment, minimize environmental contamination, and prolong pesticidal effectiveness. Targeted delivery and superior efficacy against a variety of pathogens and insect pests have been demonstrated for metallic and polymer-based nanoparticles, such as silver, copper, silica, titanium, dioxide and biopolymer composites. Nano-biopesticides are used in many different areas of agriculture. They have demonstrated potential in managing insect pests, nematodes, and fungal infections while lowering the likelihood of pest resistance, which is a significant worry with conventional pesticides. Furthermore, they play a critical role in precision agriculture and crop protection; by utilizing nano-sensors, nano-fertilizers and intelligent delivery systems, nano-biopesticides can be included into environmentally friendly farming methods that maximize input efficiency. In addition to their use in agriculture, nano-emulsion-based formulations that contain essential oils are crucial for food preservation because they provide natural substitutes for chemical preservatives and shield food items from microorganisms that cause spoiling. In a larger sense, nanobiopesticides are a tool for increasing global food security as well as an agricultural breakthrough. In light of growing population pressure, climate change, and an increase in insect outbreaks, these environmentally friendly formulations provide solutions that reduce ecological hazards while enhancing agricultural output. Furthermore, improvements in plant physiology and photosynthetic efficiency could be made possible by nanotechnology advancements, which would enhance pest control while also fostering growth. However, despite their enormous potential, a number of issues must be resolved if nano-biopesticides are to have a bright future. It is necessary to conduct a thorough assessment of the toxicity of nanoparticles, their accumulation in the environment, and their possible effects on non-target creatures, such as pollinators, beneficial microorganisms, and even human health. Many areas still lack adequate regulatory frameworks for the authorization, uniformity, and safe application of nano-biopesticides. To guarantee biocompatibility, quick biodegradability, and few adverse consequences, more study is therefore necessary. Furthermore, encouraging broad adoption will require the use of economical production techniques and raising farmer knowledge. Nano-biopesticides have the potential to be a key component of sustainable agriculture in the future. By combining nanoscale advancements with biological pest control, they provide a way to preserve or even increase crop protection while lowering reliance on dangerous chemical pesticides. In line with international objectives of environmental sustainability, food security and public health, nanobiopesticides may play a key role in Integrated Pest Management (IPM) strategies with the right regulatory oversight, interdisciplinary research, and field validation.

In summary, a paradigm shift in pest control has occurred with the switch from traditional pesticides to nano-biopesticides. These innovative formulations are extremely promising instruments for agriculture in the future since they perfectly capture the values of safety, sustainability, and precision. The appropriate development and application of nano-biopesticides can contribute to the creation of robust agricultural systems that safeguard crops, maintain ecosystems, and ensure a healthy future for both people and the environment, even though there are still questions about their long-term effects.

Conflict of interest

The authors declare no conflict of interest.

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CHAPTER 7: Microbiome–Immune Axis in Ageing:The Role of Inflammaging and Immunosenescence

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Abstract

Ageing is characterised by immunosenescence (immune decline) and inflammaging (chronic, low-grade inflammation), both of which exist in a dynamic, bidirectional relationship with age-related gut dysbiosis. As beneficial gut bacteria decline and pro-inflammatory taxa increase, a compromised intestinal barrier allows microbial translocation and impairs short-chain fatty acid (SCFA) production, fueling systemic inflammation. This review synthesises mechanistic evidence linking gut dysbiosis to immune decline via pathways such as LPS-TLR signalling and NLRP3 inflammasome activation. Finally, we evaluate therapeutic interventions targeting the microbiome–immune axis-including probiotics, dietary modifications, fecal microbiota transplantation, and senolytics-highlighting their potential to extend healthspan and lifespan in ageing populations.

Keywords: gut microbiome, aging, immunosenescence, inflammaging, dysbiosis, short-chain fatty acids, intestinal permeability, cytokines, probiotics, fecal microbiota transplantation

Introduction

The global population aged 65 years and over is projected to exceed 1.5 billion by 2050, making the biology of ageing among the most pressing areas of biomedical inquiry (World Health Organization, 2022). Ageing is not merely the passage of time but rather a fundamental biological process involving the accumulation of molecular damage, cellular dysfunction, and chronic systemic changes that ultimately increase morbidity and mortality. Among the most well-characterised aspects of biological ageing is the deterioration of immune function-a phenomenon with dual manifestations: immunosenescence (declining immune competence) and inflammaging (chronic low-grade inflammation). These two processes, once thought to be independent, are now recognised as intimately interconnected and mutually reinforcing.

In parallel, the gastrointestinal tract harbours a diverse community of approximately 10^{13}–10^{14} microorganisms-collectively termed the gut microbiome-which exerts profound influences on host immunity, metabolism, and neurological function (Qin et al., 2010). The gut microbiome is not static throughout life; it undergoes substantial compositional and functional changes during ageing, with well-documented declines in microbial diversity, loss of beneficial taxa, and expansion of potentially pathogenic species. These age-related microbial shifts-collectively described as age-related dysbiosis-are now increasingly appreciated as drivers and amplifiers of both immunosenescence and inflammaging.

The microbiome–immune axis describes the complex, bidirectional communication network through which gut microorganisms and host immune cells continuously interact. This axis operates through multiple molecular mechanisms: short-chain fatty acids (SCFAs) produced by microbial fermentation modulate regulatory T-cell (Treg) differentiation and NF-κB activity; bacterial cell wall components such as lipopolysaccharide (LPS) activate pattern-recognition receptors on immune cells; microbial metabolites including indoles and bile acids influence innate immune signalling; and the gut microbiome controls the maturation and function of gut-associated lymphoid tissue (GALT). In the context of ageing, disruption of these communication channels contributes to the progressive shift toward an inflammatory and immunodeficient phenotype.

Despite considerable progress in understanding each of these components individually, a comprehensive synthesis of how age-related dysbiosis intersects with inflammaging and immunosenescence to drive age-associated pathology remains critically needed. This review aims to address this gap by: (1) characterising age-related changes in the gut microbiome; (2) defining the mechanisms of immunosenescence and inflammaging; (3) elucidating the molecular pathways through which the microbiome modulates these immune ageing phenotypes; (4) reviewing clinical evidence and biomarkers; and (5) evaluating therapeutic strategies that target the microbiome–immune axis to promote healthy ageing.

The Ageing Gut Microbiome

Compositional Dynamics Across the Lifespan

The gut microbiome is established during the perinatal period and undergoes significant maturation through infancy, childhood, and adolescence. By adulthood, it achieves a relatively stable composition dominated by two major phyla-Firmicutes and Bacteroidetes-alongside Actinobacteria, Proteobacteria, and Verrucomicrobia (Yatsunenko et al., 2012). This compositional stability is maintained by host genetics, diet, immune factors, and environmental exposures. However, from the sixth decade of life onward, the gut microbiome exhibits consistent compositional shifts that distinguish the elderly microbiome from that of younger adults (Claesson et al., 2012).

The most consistently reported change in the ageing gut microbiome is a significant reduction in alpha-diversity-the variety of species within an individual-as measured by indices such as Shannon entropy and observed species richness. This diversity reduction has been demonstrated in multiple cross-sectional and longitudinal cohort studies across diverse populations (Biagi et al., 2010; Claesson et al., 2012). Reduced diversity is associated with decreased functional redundancy, meaning the microbiome becomes less capable of compensating for the loss of individual species, and thus more vulnerable to perturbation by antibiotics, dietary changes, or illness.

At the phylum level, the Firmicutes/Bacteroidetes ratio tends to increase with age, though findings vary by population and methodology. More consistently, Actinobacteria-particularly the genus Bifidobacterium-are markedly reduced in elderly individuals compared to younger adults (Biagi et al., 2016). Conversely, Proteobacteria, a phylum containing numerous gram-negative, LPS-producing species, expand significantly with ageing. This shift in microbiome composition has direct implications for immune function, as discussed in subsequent sections (Thevaranjan et al., 2017).

Loss of Beneficial Taxa and Functional Decline

Beyond broad compositional shifts, ageing is associated with the decline of specific beneficial species that play critical immunomodulatory and metabolic roles. *Faecalibacteriumprausnitzii*, one of the most abundant butyrate-producing bacteria in healthy adults, is consistently reduced in elderly populations and is notably absent or scarce in individuals with inflammatory bowel disease and frailty (O'Toole & Jeffery, 2015). Similarly, *Akkermansiamuciniphila*-a mucus-degrading bacterium that maintains gut barrier integrity and has been associated with metabolic health and longevity-declines with advancing age. *Lachnospiraceae* and *Ruminococcaceae*, key butyrate-producing families within the Firmicutes phylum, also decrease in representation in older microbiomes.

The functional consequences of these compositional changes are profound. SCFA production-particularly butyrate-decreases substantially in the aged gut (Table 1). Butyrate is the primary energy source for colonocytes, and its reduction compromises mucosal integrity. Moreover, butyrate functions as a histone deacetylase (HDAC) inhibitor, suppressing pro-inflammatory gene expression in immune cells. The reduction in butyrate and other SCFAs therefore directly contributes to both gut barrier dysfunction and heightened immune activation-a mechanistic link between dysbiosis and inflammaging (Rooks & Garrett, 2016; Trompette et al., 2014).

Table 1: Age-Related Changes in Gut Microbiome Composition and Their Clinical Significance.

Microbial Parameter	Young Adults	Elderly Individuals	Clinical Significance
Overall Diversity	High alpha-diversity (Shannon index)	Significantly reduced diversity	Increased vulnerability to dysbiosis
Bifidobacterium spp.	Abundant; supports immune homeostasis	Markedly reduced	Reduced SCFA, impaired gut barrier
Lactobacillus spp.	Moderate; protective	Decreased in most elderly	Less immunomodulation, ↑inflammation
Firmicutes/Bacteroidetes (F/B) Ratio	Balanced (~1–2)	Elevated ratio	Associated with obesity, metabolic disease
Proteobacteria	Low (<5%)	Significantly increased	Major source of LPS; pro-inflammatory
Akkermansiamuciniphila	Present; supports mucus layer	Reduced abundance	Compromised gut permeability
Short-Chain Fatty Acids (SCFAs)	High butyrate, propionate, acetate	Reduced, especially butyrate	↓Treg induction, ↑NF-κB activation
Faecalibacteriumprausnitzii	Abundant anti-inflammatory species	Depleted in centenarians	Key marker of intestinal health decline
Clostridiales spp.	Major butyrate producers	Reduced in frailty	Decreased colonocyte energy substrate

Note. SCFAs = short-chain fatty acids; LPS = lipopolysaccharide; NF-κB = nuclear factor kappa B; Treg = regulatory T cell. Arrows (↑/↓) indicate increase/decrease relative to young adults.

Immunosenescence: Mechanisms and Manifestations

Definition and Overview

Immunosenescence is defined as the age-associated remodelling of the immune system that results in diminished capacity to mount effective responses to novel antigens, pathogens, and vaccines, while paradoxically exhibiting increased chronic inflammatory activity (Nikolich-Žugich, 2018). This is not simply immune deficiency but rather a fundamental reprogramming-a shift in immune priorities from adaptive surveillance toward innate inflammatory activation. The concept was pioneered by Walford (1969) and substantially advanced over subsequent decades through immunological profiling of centenarians and longitudinal ageing cohorts.

Thymic Involution and T-Cell Compartment Changes

The thymus, the primary organ responsible for T-cell maturation and central tolerance, undergoes progressive involution beginning in adolescence and accelerating after the age of 40. By the seventh decade, thymic output of naïve T cells is reduced to less than 2% of its peak output (Goronzy&Weyand, 2019). This thymic involution results in a contraction of the naïve T-cell pool and an accumulation of terminally differentiated, antigen-experienced T cells-particularly CD8+ effector memory cells and late-differentiated effector cells (TEMRA). These senescent CD8+ T cells are characterised by shortened telomeres, downregulation of the costimulatory molecules CD27 and CD28, and expression of inhibitory receptors such as PD-1 and KLRG1.

The contraction of the T-cell receptor (TCR) repertoire-a consequence of both thymic decline and clonal expansion-reduces the diversity of antigen recognition, leaving elderly individuals vulnerable to novel pathogens. This is dramatically illustrated by the increased susceptibility and mortality from influenza, SARS-CoV-2, and other emerging infections in older populations. Additionally, cytomegalovirus (CMV) seropositivity, near-universal in older adults, drives extensive clonal expansion of CMV-specific T cells that fills immunological space and further restricts naïve T-cell availability-a phenomenon termed the immune risk profile (Fulop et al., 2018).

B-Cell and NK-Cell Dysfunction

The B-cell compartment also undergoes significant age-related remodelling. The ratio of naïve to memory B cells shifts toward memory cells, and B-cell repertoire diversity contracts due to reduced bone marrow B-cell lymphopoiesis. Age-related intrinsic B-cell defects include reduced expression of activation-induced cytidine deaminase (AID), impaired class switch recombination, reduced somatic hypermutation efficacy, and diminished affinity maturation-resulting in lower-quality antibody responses to vaccination and infection (Haynes &Maue, 2009). Natural killer (NK) cells, critical effectors of antiviral and antitumour immunity, also decline in function with age, showing reduced cytotoxicity per cell despite often-increased numbers in circulation.

Innate Immune Alterations

Innate immune cells including dendritic cells (DCs), monocytes, macrophages, and neutrophils exhibit significant functional impairments with ageing. Plasmacytoid DCs (pDCs) show reduced production of type I interferons in response to TLR7 and TLR9 stimulation-a critical early antiviral response. Monocytes display impaired phagocytosis, reduced antigen presentation capacity, and polarisation toward pro-inflammatory M1 phenotypes (Panda et al., 2010). Neutrophil chemotaxis, phagocytosis, and NET formation are all diminished in elderly individuals, contributing to impaired bacterial clearance and increased susceptibility to secondary bacterial infections following viral illness.

Inflammaging: The Chronic Inflammatory State of Ageing

Defining Inflammaging

The term "inflammaging" was coined by Franceschi et al. (2000) to describe the chronic, low-grade, sterile pro-inflammatory state that develops progressively with ageing. Unlike acute inflammation-which is transient, protective, and resolves upon elimination of the inciting stimulus-inflammaging is persistent, low-amplitude, and systemic, occurring in the absence of overt infection. It is characterised by chronically elevated circulating levels of pro-inflammatory mediators including IL-6, IL-1β, TNF-α, C-reactive protein (CRP), and interferon-gamma (IFN-γ), alongside elevated soluble CD14 (sCD14) and lipopolysaccharide-binding protein (LBP)-biomarkers of microbial translocation (Franceschi&Campisi, 2014).

Critically, inflammaging does not reflect a failure of immune regulation per se, but rather a sustained activation of innate immune pathways by multiple simultaneous stimuli-many of which are endogenous. These include: (1) senescence-associated secretory phenotype (SASP) from accumulating senescent cells; (2) mitochondrial damage-associated molecular patterns (DAMPs) released from dysfunctional mitochondria; (3) endogenous retroelements and self-DNA activating the cGAS-STING pathway; (4) nuclear factor kappa B (NF-κB) activation driven by oxidative stress; and (5) microbial translocation from the ageing gut (De la Fuente & Miquel, 2009).

The NLRP3 Inflammasome and Cytokine Storm in Ageing

The NLRP3 inflammasome-a multiprotein cytosolic complex that processes pro-IL-1β and pro-IL-18 into their active forms-plays a central role in inflammaging. With advancing age, NLRP3 activation thresholds are lowered by mitochondrial reactive oxygen species (ROS), uric acid crystals, cholesterol crystals, and gut-derived LPS. Chronic NLRP3 activation sustains IL-1β and IL-18 secretion, contributing to the systemic inflammatory milieu characteristic of inflammaging. The NLRP3 inflammasome also drives pyroptosis-an inflammatory form of programmed cell death-which further amplifies local and systemic inflammation (Coppé et al., 2010). Importantly, gut microbiota-derived signals, particularly LPS from Proteobacteria and bacterial flagellin, are potent NLRP3 activators, directly linking gut dysbiosis to inflammaging.

The Senescence-Associated Secretory Phenotype (SASP)

Cellular senescence-the irreversible cell-cycle arrest triggered by DNA damage, telomere shortening, oncogene activation, or oxidative stress-is accompanied by the SASP: a complex secretome that includes cytokines (IL-6, IL-8, GRO-α), matrix metalloproteinases (MMPs), growth factors, and chemokines. While SASP serves homeostatic functions including wound healing and tumour suppression in young tissues, its chronic persistence in aged tissues amplifies local and systemic inflammation. Senescent cells accumulate in the gut epithelium, liver, adipose tissue, and immune organs during ageing, and their SASP directly modifies adjacent immune cell behaviour, driving further inflammation and immune dysfunction (Coppé et al., 2010).

The Microbiome–Immune Axis: Bidirectional Communication

Mechanisms of Immune Regulation by the Gut Microbiome

The gut microbiome communicates with the host immune system through multiple parallel mechanisms that operate locally at the gut mucosa and systemically via microbial metabolites and structural components that translocate across the epithelial barrier. Locally, the gut microbiome continuously stimulates pattern-recognition receptors (PRRs) on intestinal epithelial cells (IECs) and lamina propria immune cells-including TLR2, TLR4, TLR5, and TLR9-to maintain homeostatic immune tone. This tonic signalling is essential for normal gut-associated lymphoid tissue (GALT) development and secretory IgA (sIgA) production. Loss of beneficial microbial signals during ageing thus impairs mucosal immunity even before pathological dysbiosis becomes established (Kau et al., 2011).

At the systemic level, microbial metabolites-most prominently SCFAs-enter the portal circulation and directly modulate immune cell function in the liver, spleen, bone marrow, and adipose tissue. Butyrate and propionate activate G-protein coupled receptors (GPR41, GPR43, GPR109a) on immune cells, suppressing NF-κB activation, promoting FOXP3+ Treg differentiation, and inhibiting histone deacetylation to repress inflammatory gene expression. The role of SCFAs in maintaining immune tolerance is therefore critically dependent on microbiome composition-a relationship disrupted by age-related dysbiosis (Rooks & Garrett, 2016).

Gut Barrier Integrity, LPS Translocation, and Endotoxemia

The intestinal epithelial barrier-comprising a single layer of epithelial cells connected by tight junction proteins including occludin, claudins, and zonula occludens-1 (ZO-1)-serves as the critical interface between the microbial-rich luminal environment and the systemic circulation. Ageing compromises barrier integrity through multiple mechanisms: reduced expression of tight junction proteins, decreased mucus production by goblet cells, impaired epithelial renewal due to stem cell dysfunction, and direct effects of dysbiotic microbiota on barrier function. A. muciniphila, whose abundance declines with age, plays a central role in maintaining the mucus layer; its loss correlates with increased epithelial permeability.

The consequence of barrier dysfunction is increased translocation of microbial products-particularly LPS from gram-negative Proteobacteria-into the systemic circulation, a state termed metabolic endotoxemia. LPS binds TLR4 on monocytes, macrophages, dendritic cells, and hepatocytes, activating the MyD88/NF-κB and TRIF/IRF3 signalling cascades and driving sustained production of IL-6, TNF-α, and IL-1β (Thevaranjan et al., 2017). Circulating LPS concentrations, measured as sCD14 or LPS activity, are consistently elevated in elderly individuals and correlate with markers of frailty, cognitive decline, and cardiovascular risk-providing a direct mechanistic link between gut dysbiosis and systemic inflammaging.

Tryptophan Metabolism and the Gut–Immune–Brain Axis

The essential amino acid tryptophan is metabolised by gut microbiota along three major pathways: the kynurenine pathway (mediated by indoleamine-2,3-dioxygenase, IDO1), the serotonin pathway, and the indole pathway. In the context of ageing, IDO1 activity increases-driven by chronic IFN-γ elevation-shifting tryptophan metabolism toward immunosuppressive kynurenine metabolites. While this initially serves to dampen excessive inflammation, chronic kynurenine elevation is paradoxically associated with neurotoxicity, immune dysregulation, and age-related depression. Conversely, indole production by gut microbiota activates the aryl hydrocarbon receptor (AhR) in intestinal immune cells, promoting IL-22 production, barrier repair, and anti-inflammatory immune programming. Age-related loss of indole-producing bacteria thus contributes to mucosal immune dysfunction (Sonnenburg&Bäckhed, 2016).

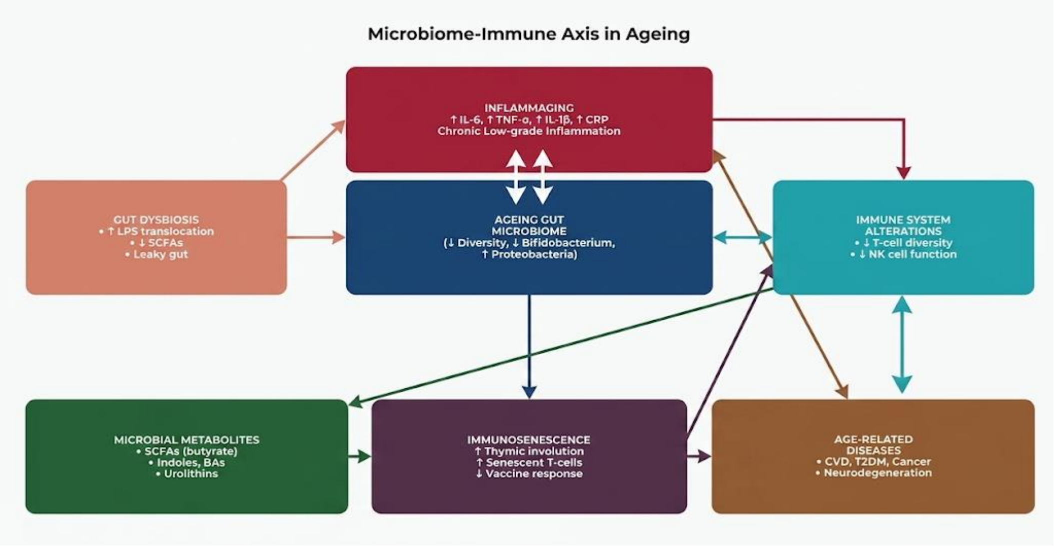

Figure 1. Schematic representation of the Microbiome–Immune Axis in Ageing. The figure illustrates the bidirectional interactions between age-related gut dysbiosis, intestinal permeability, microbial metabolites, inflammaging, immunosenescence, and age-related diseases. SCFAs = short-chain fatty acids; LPS = lipopolysaccharide; BAs = bile acids; IL = interleukin; TNF-α = tumour necrosis factor-alpha; CRP = C-reactive protein. Arrows indicate the direction of influence.

Microbiome-Mediated Pathways Linking Dysbiosis to Inflammaging and Immunosenescence

The SCFA–Treg Axis and Loss of Immune Tolerance

Regulatory T cells (Tregs) expressing the transcription factor FOXP3 are essential for maintaining peripheral immune tolerance and suppressing excessive inflammatory responses. The differentiation, stability, and function of peripheral Tregs are critically dependent on microbial-derived SCFAs-particularly butyrate and propionate-which enhance FOXP3 expression through HDAC inhibition and GPR43/GPR109a signalling. In aged individuals, the reduction in SCFA-producing bacteria leads to a quantitative and qualitative deficiency of Tregs, impairing immune tolerance mechanisms and facilitating the chronic inflammatory activation characteristic of inflammaging (Rooks & Garrett, 2016). Animal models of germ-free and dysbiotic ageing consistently demonstrate that SCFA supplementation can partially restore Treg populations and reduce systemic inflammation.

Microbiome-Induced NLRP3 Inflammasome Activation

Gut-derived signals are potent activators of the NLRP3 inflammasome in macrophages and other innate immune cells. LPS from translocated gram-negative bacteria provides the priming signal (signal 1) for NLRP3 expression via TLR4/NF-κB. Additional signals from ATP released by dying epithelial cells, uric acid crystals, or microbially modified lipids serve as the activation signal (signal 2), triggering NLRP3 oligomerisation and caspase-1-dependent IL-1β processing. In aged tissues, the combination of increased gut permeability, elevated circulating LPS, and heightened cellular stress creates a permissive environment for constitutive NLRP3 activation-perpetuating the inflammatory cycle of inflammaging.

Bile Acid Dysmetabolism

Bile acids (BAs)-synthesised by the liver from cholesterol and modified by gut bacteria-are emerging as critical immune regulators. Primary bile acids (cholic acid and chenodeoxycholic acid) are deconjugated and converted to secondary bile acids (deoxycholic acid and lithocholic acid) by specific commensal bacteria. Secondary BAs activate the Takeda G-protein-coupled receptor 5 (TGR5) on macrophages, inhibiting NF-κB and reducing pro-inflammatory cytokine production. Age-related dysbiosis-particularly the loss of bile salt hydrolase (BSH)-expressing Lactobacillus and Bifidobacterium species-disrupts secondary BA metabolism, impairing these anti-inflammatory BA-TGR5 signals. The resulting shift toward a pro-inflammatory BA profile contributes to elevated hepatic inflammation and systemic inflammaging (Walker & Lawley, 2013).

Table 2: Key Features of Immunosenescence and Inflammaging with Microbiome Linkages.

Feature	Immunosenescence	Inflammaging	Microbiome Link
Definition	Age-related decline in immune function	Chronic low-grade sterile inflammation	Both driven by gut dysbiosis
Key Immune Cell Changes	↓Naïve T/B cells; ↑senescent CD8+ T cells; Thymic involution	↑M1 macrophage polarization; ↑SASP	↓SCFAs → ↓Treg; LPS activates macrophages
Cytokine Profile	↓IL-2, IFN-γ; ↓vaccine-induced Ab titers	↑IL-6, IL-1β, TNF-α, IL-8, IL-18	Gut-derived LPS activates NF-κB pathway
Innate Immunity	↓NK cell cytotoxicity; Impaired phagocytosis	Persistent TLR activation; NLRP3 inflammasome	Microbiota activates TLR4/TLR5 signaling
Adaptive Immunity	↓TCR diversity; ↑CMV-driven clonal expansion	Chronic antigen stimulation; ↑Tfh dysfunction	Microbial antigens drive chronic activation
Serum Biomarkers	↓DHEA; ↓IGF-1; ↑p16INK4a; ↑p21	↑CRP, ↑IL-6, ↑sCD14, ↑LBP	sCD14, LBP reflect microbial translocation
Gut Barrier Association	↓IgA secretion; ↓mucosal immunity	↑Intestinal permeability (leaky gut)	Tight junction protein loss (occludin, ZO-1)
Disease Associations	Recurrent infections; ↑cancer incidence	CVD, T2DM, Alzheimer's, sarcopenia	Microbiome dysbiosis common precursor

Clinical Evidence and Biomarkers

Microbiome Signatures in Centenarians and Long-Lived Individuals

Studies of centenarians-individuals who have surpassed 100 years of age with relatively preserved health-provide compelling evidence for the role of microbiome composition in healthy ageing. Biagi et al. (2016) demonstrated that Italian centenarians harbour a distinctive microbiome enriched with Christensenellaceae, Akkermansia, and Bifidobacterium compared with elderly controls in their 70s–90s. These taxa are associated with lean body composition, reduced inflammatory markers, and enhanced immune function. Moreover, semi-supercentenarians (aged 105–109) showed even higher enrichment of Akkermansia and anti-inflammatory taxa, suggesting a progressive microbiome selection for longevity-associated species.

Conversely, frail elderly individuals show the most pronounced dysbiosis: reduced Lactobacillus, Bifidobacterium, and butyrate producers; increased Proteobacteria; and elevated fecal calprotectin (an inflammatory marker). The degree of microbiome diversity correlates inversely with CRP, IL-6, and frailty indices in multiple European cohort studies (Claesson et al., 2012). These associations suggest the microbiome is not merely a bystander in the ageing process but an active determinant of inflammatory and immune status.

Key Inflammatory and Microbiome-Related Biomarkers

Circulating biomarkers that reflect the microbiome–immune interface in ageing include: (1) sCD14-a receptor shed by monocytes upon LPS stimulation, reflecting microbial translocation; (2) LPS-binding protein (LBP)-elevated in conditions of increased gut permeability; (3) intestinal fatty acid-binding protein (I-FABP)-a marker of enterocyte damage; (4) zonulin-a protein that modulates tight junction opening; and (5) serum IL-6, which integrates both SASP-derived and microbiome-driven inflammatory signals. These biomarkers, combined with microbiome profiling by 16S rRNA sequencing or shotgun metagenomics, are increasingly used in ageing research to stratify inflammatory risk and evaluate therapeutic interventions.

Therapeutic Strategies Targeting the Microbiome–Immune Axis

Given the central role of microbiome dysbiosis in driving inflammaging and immunosenescence, restoration of a health-associated microbiome composition represents an attractive therapeutic strategy for promoting healthy ageing. Table 3 summarises the major evidence-based interventions targeting the microbiome–immune interface.

Table 3: Therapeutic Strategies Targeting the Microbiome–Immune Axis in Ageing.

Intervention	Mechanism	Evidence Summary	Limitations
Probiotics (Lactobacillus, Bifidobacterium)	Restore commensal balance; produce SCFAs; enhance IgA	RCTs show ↓IL-6, ↑NK cell activity, improved vaccine response in elderly (Makino et al., 2010; Malaguarnera et al.,	Strain-specific; transient colonization; variable efficacy

		2012)	
Prebiotics (FOS, GOS, Inulin)	Selectively feed beneficial bacteria; increase SCFA production	Galacto-oligosaccharides ↑Bifidobacterium, ↑NK cells in older adults (Vulevic et al., 2015)	GI side effects; dose-dependent response
Mediterranean Diet	Polyphenols, fiber → anti-dysbiosis; ↓oxidative stress	NU-AGE trial showed diet reduces inflammatory biomarkers; enhances Lactobacillus in elderly (Ghosh et al., 2020)	Adherence challenges; cultural variation
Fecal Microbiota Transplantation (FMT)	Direct transfer of young/healthy donor microbiome to recipient	Animal studies show reversal of immunosenescencemarkers (Bárcena et al., 2019); human trials limited	Safety concerns; donor screening; ethical issues
Postbiotics (Butyrate supplementation)	Directly inhibit NF-κB; HDAC inhibitor; induce Tregs	Sodium butyrate ↓IL-6, ↑mucosal immunity in preclinical and early clinical studies	Bioavailability; palatability; dose optimization needed
Senolytics (Dasatinib + Quercetin)	Clear senescent cells → reduce SASP → indirect microbiome benefit	Early trials show ↓senescent cell burden; microbiome effects under investigation	Limited long-term data; organ toxicity potential
Exercise Interventions	↑Butyrate-producing bacteria; ↓systemic inflammation; improves gut motility	Regular aerobic exercise ↑Lachnospiraceae, ↑Akkermansia, ↓CRP in elderly cohorts	Adherence issues in frail elderly populations

Note. RCT = randomised controlled trial; FOS = fructo-oligosaccharides; GOS = galacto-oligosaccharides; FMT = fecal microbiota transplantation; NK = natural killer; CRP = C-reactive protein; SASP = senescence-associated secretory phenotype.

Probiotics and Synbiotics

Probiotic supplementation with Lactobacillus and Bifidobacterium strains has been evaluated in multiple randomised controlled trials (RCTs) in elderly populations. Makino et al. (2010) demonstrated that daily consumption of Lactobacillus delbrueckii ssp. bulgaricus OLL1073R-1 fermented yoghurt reduced the incidence and duration of upper respiratory infections in nursing home residents, associated with enhanced NK cell activity. Malaguarnera et al. (2012) reported that Bifidobacterium longum supplementation combined with fructo-oligosaccharides reduced IL-8 and TNF-α in elderly patients with Alzheimer's disease, illustrating the gut–brain–immune axis. Synbiotics-combinations of probiotics and prebiotics-show synergistic benefits and represent a promising approach for sustained microbiome modulation in ageing populations.

Dietary Interventions

The Mediterranean diet-characterised by high consumption of vegetables, fruits, legumes, whole grains, fish, olive oil, and moderate wine intake-has been consistently associated with reduced inflammaging biomarkers and preserved immune function in older adults. The NU-AGE multicentre European trial demonstrated that one year of Mediterranean diet intervention significantly altered the gut microbiome of elderly participants, increasing Lachnospiraceae, Ruminococcaceae, and Akkermansia while decreasing pro-inflammatory taxa; these changes correlated with reduced IL-17, IL-8, and improved physical function scores (Ghosh et al., 2020). The mechanistic basis involves increased dietary fibre driving SCFA production, polyphenol-mediated Akkermansia expansion, and omega-3 fatty acid-dependent resolution of inflammation.

Fecal Microbiota Transplantation and Future Directions

FMT-the transfer of processed fecal material from a healthy young donor to an aged recipient-represents the most direct approach to restoring a youthful microbiome composition. Bárcena et al. (2019) demonstrated in progeroid mouse models that FMT from young donors restored intestinal microbiome diversity, reduced markers of DNA damage and inflammation, extended lifespan, and improved healthspan parameters. Human FMT studies in ageing are limited to safety and feasibility data, but preliminary evidence suggests potential benefits in reducing frailty-associated dysbiosis. Standardisation of donor selection, preparation protocols, and regulatory frameworks remains a critical challenge.

Future Directions

Several critical research gaps remain in understanding the microbiome–immune axis in ageing. First, most current evidence is correlative rather than mechanistic; larger, longitudinal intervention studies are needed to establish causality between microbiome modulation and immune ageing outcomes. Second, the heterogeneity of the ageing population-influenced by genetics, lifestyle, geographic location, comorbidities, and medications (particularly antibiotics and proton pump inhibitors)-demands personalised microbiome-based therapeutic approaches. Third, the development and validation of comprehensive biomarker panels integrating microbiome composition, metabolomics, and immune profiling will be essential for patient stratification and monitoring therapeutic efficacy.

Advances in multi-omics technologies-including metagenomics, metatranscriptomics, metabolomics, and single-cell immunophenotyping-are beginning to reveal previously inaccessible layers of microbiome–immune interaction. The integration of these data streams with machine learning approaches holds promise for identifying microbial and immune signatures that predict trajectories of healthy versus pathological ageing. Furthermore, the development of next-generation probiotics comprising single strains or defined consortia of health-promoting bacteria (e.g., A. muciniphila, F. prausnitzii) produced under GMP conditions represents a promising frontier. Pharmacological modulation of microbiome–immune pathways-including AhR agonists, TGR5 agonists, and GPR43 activators-may also offer novel therapeutic targets for inflammaging.

The intersection of senolytics and microbiome modulation is particularly intriguing. By clearing senescent cells and reducing SASP, senolytics may indirectly improve intestinal barrier function and reduce the inflammatory milieu that drives dysbiosis-creating a virtuous cycle of anti-ageing benefits. Combination strategies that target both the microbiome and cellular senescence simultaneously warrant systematic investigation in preclinical and clinical settings.

Conclusion

The microbiome–immune axis represents one of the most dynamic and therapeutically tractable interfaces in the biology of ageing. Age-related gut dysbiosis-characterised by reduced microbial diversity, loss of beneficial taxa, increased Proteobacteria, and impaired SCFA production-directly contributes to the dual phenomena of inflammaging and immunosenescence through mechanisms involving gut barrier dysfunction, LPS translocation, NLRP3 inflammasome activation, SCFA–Treg axis disruption, tryptophan dysmetabolism, and bile acid dysregulation. These processes interact in complex, mutually reinforcing loops that accelerate immune decline and drive age-related pathology including cardiovascular disease, type 2 diabetes, neurodegenerative disorders, and cancer.

Current evidence strongly supports the view that the gut microbiome is not merely a bystander of ageing but an active participant in determining the pace and trajectory of immune ageing. Interventions that restore microbiome homeostasis-dietary modification, probiotics, prebiotics, postbiotics, and FMT-show significant promise for attenuating inflammaging and preserving immune function in older adults. As the field moves toward precision geroscience, integrating microbiome data with immune profiling and multi-omics approaches will enable more targeted, personalised strategies for extending healthspan. The microbiome–immune axis thus stands as a compelling target for the next generation of anti-ageing therapeutics.

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CHAPTER 8: Echoes of CGG: When Repeats Rewrite Lives From Mutation to Manifestation in Fragile X Syndrome

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Abstract

Fragile X Syndrome (FXS) is the most common inherited cause of intellectual disability and the second most prevalent genetic cause after Down syndrome. It is an X-linked disorder caused by an abnormal expansion of CGG trinucleotide repeats (≥200) in the 5′ untranslated region of the FMR1 gene at Xq27.3. This expansion triggers DNA hypermethylation, histone modifications, and altered transcriptional regulation, leading to silencing of FMR1 and deficiency or absence of fragile X mental retardation protein (FMRP), a key regulator of synaptic function and cognitive development. FXS is associated with intellectual disability, autistic-like behaviors, anxiety, mood instability, aggression, sensory hypersensitivity, sleep disturbances, and distinct physical features such as an elongated face and prominent ears. Males typically shows more severe symptoms due to the absence of a second X chromosome, whereas females often have milder, symptoms due to the presence of a second, typically functioning, X chromosome. Animal models—including Fmr1 knockout mice, zebrafish, and drosophila have clarified molecular mechanisms, behavioral phenotypes, and therapeutic targets. Globally, FXS affects approximately 1 in 4,000 males and 1 in 8,000 females, with regional variations. India reports high prevalence rates, while the Colombian district of Ricaurte exhibits exceptionally high prevalence, underscoring the influence of genetic clustering and the need for targeted regional research. This review integrates molecular, clinical, and epidemiological perspectives to clarify the complex neurogenetic basis of FXS, emphasize early diagnosis, and evaluate emerging therapies. Understanding its pathogenesis, prevalence, and clinical spectrum is essential for timely detection, personalized interventions, and improving neurodevelopmental and life outcomes for affected individuals.

Keywords: Fragile X Syndrome (FXS), inherited cause, intellectual disability, X-linked disorder, FMR1 gene, DNA hypermethylation, histone modifications, altered transcriptional regulation, silencing, fragile X mental retardation protein (FMRP), synaptic function, cognitive development, Fmr1 knockout mice, zebrafish, drosophila, genetic clustering, neurogenetic basis.

Introduction

Fragile X Syndrome (FXS) is a non-mendelian inherited disease via the X chromosome, first described in 1943 by Martin and Bell. It is actually the most frequent inherited cause of intellectual disability and the second most frequent cause after Down syndrome. It is a result of an abnormal increase in the size of the trinucleotide CGG repeats in the 5' UTR within the fragile X mental retardation 1 gene (FMR1) at locus Xq27.3. It is a dynamic mutation with growth of the CGG repeat in every generation progressing from the premutation (PM) range of 55 to 200 repeats and enlarging to a full mutation (FM) when transmitted by a women to her offspring [Bagni C et al, 2012] .This mutation leads to a deficiency or lack of FMR1 protein (FMRP) needed for normal cognitive development and synaptic function [Fazna A & Hagerman R.J. et al, 2024]. Autistic-like behaviors are common in persons with FXS and include flapping of hands, biting of hands, avoidance of eye contact, tactile defensiveness, and hyperarousal to sensory input. The affective and behavioral profile in females with FXS is typically variable. Females with the full mutation are at risk for social anxiety, shyness, social avoidance, withdrawal, language impairment, mood lability, and depression. [Garber K.B. et al, 2015].

The FMR1 gene is more prevalent in premutations (55–200 CGG repeats) than in full mutations. Consistent frequency of the premutation was 1 in 130–250 in females and 1 in 250–800 in males, and this has been approximated from various demographic studies. [Seltzer M.M. et al, 2012][Fazna A & Hagerman R.J. et al, 2024]. Female PM carriers have a chance of passing an expanded CGG repeat to the child. In oogenesis (formation of the egg), the CGG repeat is expanded, and this may lead to the child having a full mutation, hence resulting in FXS [Nolin et al, 1994]. "Gray zone" or intermediate alleles of the FMR1 gene (45–54 CGG repeats) can be regarded as precursors to premutation alleles [Protic D.D. et al, 1994]. For PM alleles with >99 CGG repeats, there is a very high chance of PM-to-FM transition, i.e., very near to 100%. PM individuals possess a normal IQ level, while it is observed that female PM carriers have a high chance of having a child with FXS. It has also been observed that PM neurons exhibit premature cell death in culture, with increased susceptibility to toxins [Yrigollen C.M. et al, 2014].

There exist several animal models that are utilized to investigate Fragile X: Fmr1 knock-out (KO) rat and mice model, Drosophila melanogaster model of fragile X, and Fmr1 KO zebrafish [Dahlhaus R, 2018] [Protic D.D. et al, 1994]. Over 20 years ago, the Fmr1 KO mouse model was created in order to assist in the comprehension of FXS. Studies in the mouse model have shown that the Fmr1 gene has a role in plasticity and synapse formation, and this has provided potential targets for drug development for FXS [Protic D.D. et al, 1994]. Fmr1 KO zebrafish are also a good model frequently employed to investigate ASD and social behavior because they aggregate in shoals. Experiments in the zebrafish replicate the Fmr1 experiments in transgenic mice. In addition, these behavioral traits like anxiety and hyperactivity seen in FXS and ASD individuals were also noted in the zebrafish model [Kim L et al, 2014]. In the model Drosophila fragile X, the one ortholog of FMR1, dfmr1, has been knocked out and leads to the lack of dFMRP. This model has been highly productive when it comes to improving our understanding of FXS pathogenesis. It shows a number of pertinent phenotypes, such as abnormalities in the circadian output pathway, sleep issues, memory impairments in the conditioned courtship and olfactory conditioning models, social interaction impairments (peer and naïve courtship), and impairments in neural growth [Protic D.D. et al, 1994].

This review paper focuses on the molecular mechanism, clinical features, and a case study in real-life of Fragile X Syndrome. This most prevalent inherited cause of intellectual disability is a fascinating crossroads of genetics, neurodevelopment, and epidemiology. It is hoped that this paper will de-mystify the FMR1 gene silencing underlying phenomenon and its effects on synaptic function as well as illustrate the symptom range from cognitive impairments to behavioral difficulties. Finally, this review, article aims to enhance own understanding and which also enables fellow students to better understand the intricacies of genetic disorders.

Mechanism

The full mutation (FM) of the FMR1 gene is caused by the expansion of CGG trinucleotide repeats (≥ 200) in the 5’ untranslated region (UTR) of the gene. This occurs due to the meiotic instability of specific alleles. [Protic D.D. et al, 1994]. The body perceives the FMR1 gene having 5-44 CGG repeats as normal, which is, in fact, the result of conservative recombination. The FMR1 gene silencing due to hypermethylation, histone alteration and transcriptional regulation fall into three categories.

As a result, gene expression is ceasided due to hypermethylation, gene silencing, and a reduction or absence of FMRP. [Saldarriaga W et al, 2014]. In FXS, FMR1 genes are devoid of active transcription due to increased methylation of cytosines roughly 1-kb upstream from the CGG repeats, inclusive of the FMR1 promoter.Normal alleles do experience methylation in the FMR1 promoter region, but not near the CGG repeat sequence, which appears to be a “boundary” in the normal allele that halts methylation from spreading further. This boundary is absent in FM alleles, and the methylation of the cytosines occurs upstream of the CGG repeat, commencing around the thirteenth week of fetal development [Malter H.E. et al, 1997]. In certain instances, alleles which are partially or fully unmethylated (UFM) do not exist. These alleles possess more than 200 CGG repeats. Female premutation carriers are capable of transmitting the genetically expanded CGG repeat to their children. During oogenesis, the CGG repeat is capable of undergoing expansions that may result in a fully mutated allele in the child, leading to fragile X syndrome (FXS). The likelihood of expansion approaches a full mutation threshold correlates positively with the CGG repeat count present in the mother's premutation status. [Nolin S.L. et al, 1994].

In relation to FM alleles, the FMR1 gene is silenced epigenetically through specific modifications to the gene's histones. There is under-acetylation of histones H3 and H4, which diminishes the accessibility of the chromatin. There is also an increase of Lysine 9 (K9) methylation, known to be a repressive mark of heterochromatin, and a decrease of Lysine 4 (K4) methylation which is a mark of transcriptional activation. In combination, these changes form a tightly folded chromatin structure that hinders transcription factors from reaching the FMR1 promoter region. This form of epigenetic alteration effectively silences FMR1 expression, which, in turn, FMRP is absent. [Kumari D & Usdin K et al, 2001] The regulation of transcription of the FMR1 gene involves a sophisticated structural promoter system which is composed of elements E-boxes which bind transcription factors, USF1 and USF2, and GC-boxes, which are recognized by Sp1 and Sp3. [Kumari D et al, 2005].

In addition, some initiator-like motifs situated roughly 130 nucleotides upstream of the CGG repeat region help in the initiation of transcription. Normally, these elements are normal alleles help in pronounced transcription in cooperation. Yet, with expansion of CGG repeats in the full mutation alleles, the transcriptional start site moves upstream, independent of the spatial arrangement of some of the crucial regulatory elements. This phenomenon changes the dynamics of transcription factor binding and the efficiency of the promoter which enhances gene silencing and thus, the development of Fragile X syndrome via reduced FMRP.[Beilina A et al, 2004].

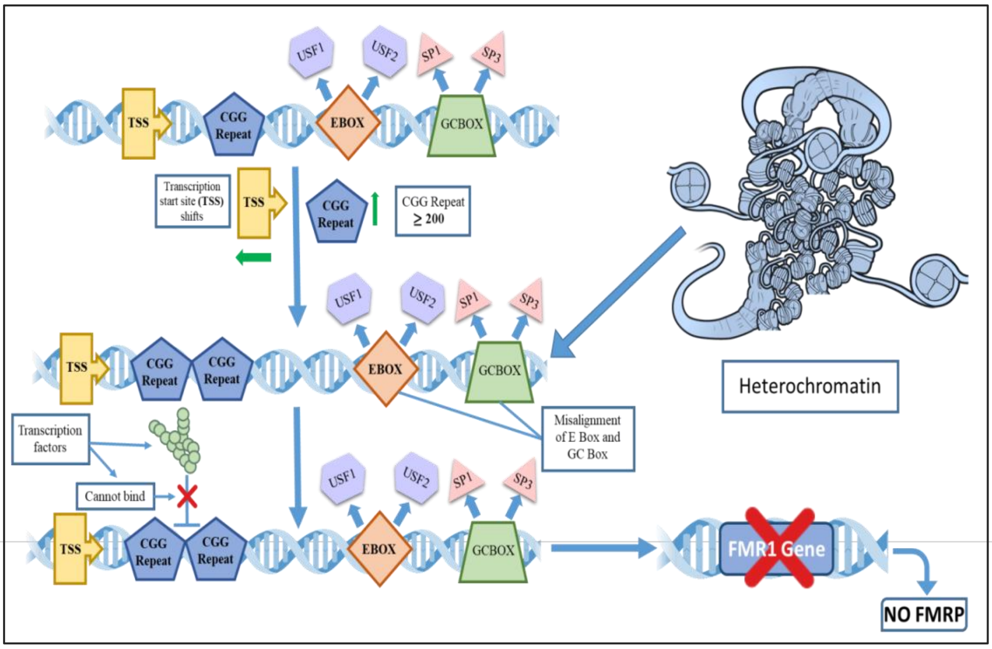


FIG: 1. HYPERMETHYLATION 2. HISTONE MODIFICATION

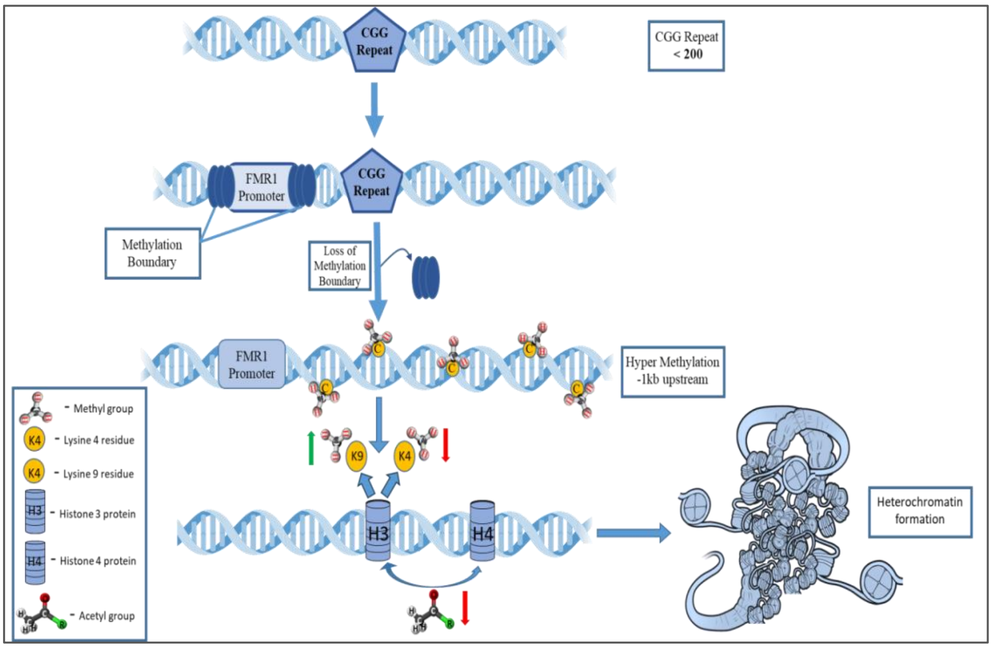


FIG: 2. TRANSCRIPTIONAL MODIFICATION

Symptoms

Anxiety disorders are particularly prevalent among individuals with FXS, with 70 to 80% exhibiting some form of anxiety. For such cases, selective serotonin reuptake inhibitors (SSRIs) are prescribed and have been shown to work very well, such as with sertraline. These medications enhance serotonin levels in the synapse, which is a crucial mood stabilizer in the central nervous system, by acting on the presynaptic serotonin reuptake terminals. [Hess L.G. et al, 2016].

Explosive temper tantrums,self-injurious behaviors, and aggression are prevalent in FXS and tend to emerge during the teenage years. Antipsychotics of atypical class such as risperidone and aripiprazole are effective in treating the irritability and aggression as well as the meltdowns which accompany the disorder. These medications work through dopaminergic and serotonergic neural pathways. [Hagerman R.J. et al, 2009]. 30-60% of individuals with FXS are also affected by obesity, which is caused by compulsive eating behaviors that develop as a coping mechanism to anxiety and irritability. [McLennan Y et al, 2011].

Like other disorders, FXS is also associated with sleep disorder. The prevalence of sleep difficulties among patients with FXS is 27% to 77%. For these individuals, melatonin is the most effective sleep aid and is the number one treatment option. Melatonin is a neurohormone released and synthesized predominantly by the pineal gland, particularly at night. It is known for regulating the body's circadian rhythms and is known for promoting sleep by acting on melatonin receptors located at the suprachiasmatic nucleus. [Srinivasan V et al, 2009] [Protic D.D. et al, 2022].

Some other symptoms associated with FXS are—narrowed and elongated facial features, large protruding ears, stereotypical hand movements, aversive response to tactile stimuli. [Maes B et al, 2000].

Case Study

A 3-year and 6-month-old boy was diagnosed with FXS at 15 months, where he was found to have a full mutation with 250–650 CGG repeats. He was born with feeding challenges such as poor latching, vomiting, and dysphagia associated with solid foods. His weight gain was slow, falling within the 3rd to 5th percentiles. He also displayed hypotonia, and motor milestones were delayed. He showed early behavioral concerns including stereotypic movements associated with heightened sensory sensitivities, a curtailed attention span, and difficulties engaging in eye contact and social interactions.MRI, ECG, and other lab work were within normal limits.

His parents started him on a high-CBD oral paste (CBD+; 50 mg/day) at 15 months which resulted in rapid behavioral, social, and feeding improvements within a week. He began early intervention with speech and occupational therapy a month later. Over the following three months, he demonstrated improved motor coordination, enhanced vocalizations, decreased anxiety, diminished repetitive behaviors, improved eye contact, and reduced anxiety. His weight improved to the 15th percentile by 19 months. For further support on language acquisition, dose 2.5 mg/day of sertraline was added to the regimen.

Some of the other areas of improvement noted were walking, self-feeding, enhanced social interaction, reduced sensory challenges, and improved communication skills. His weight increased to the 25th percentile, and he continued to respond positively to the combination of CBD+ with an intensive therapy schedule. CBD+ was paired with minocycline 25 mg/day two months later. This combination, however, worsened anxiety, sleep, and behaviors. By age three, he experienced problems with attention and transitions which persisted despite therapy and increased sertraline dosing to 4mg/day. After a multidisciplinary team discussion, CBD+ was reinstated, which led to renewed improvements in anxiety, meltdowns, and sleep. He successfully transitioned to a small preschool while continuing CBD+, sertraline, minocycline, and early intervention, demonstrating steady developmental progress. [Tartaglia N et al, 2019].

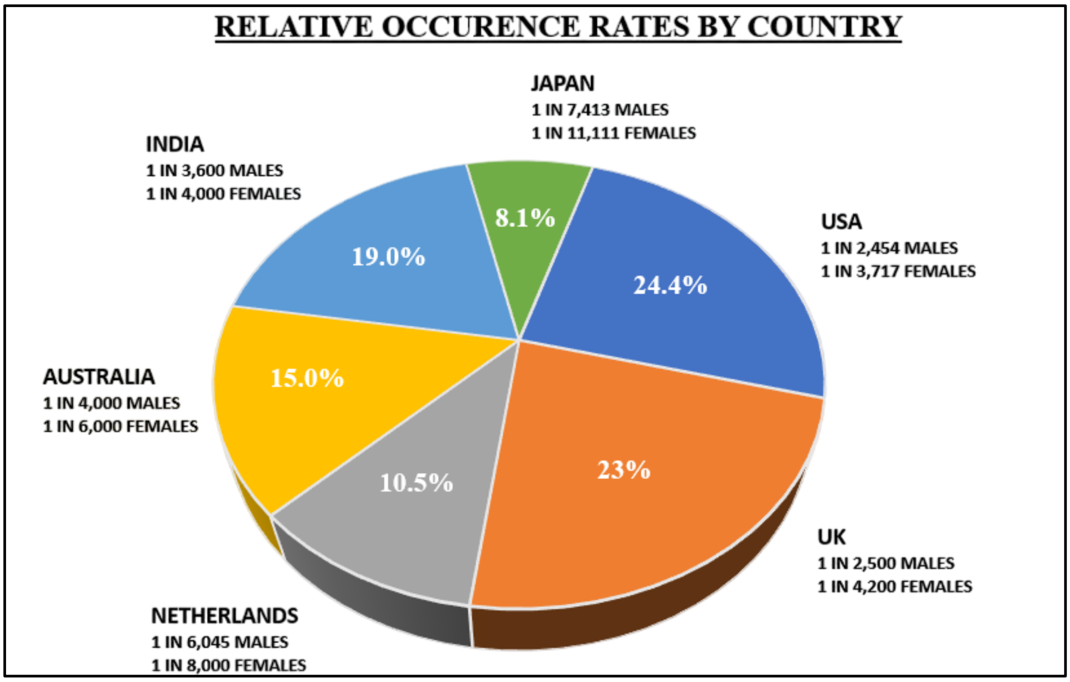
Statistics

Worldwide prevalence of Fragile X Syndrome (FXS) is extremely heterogeneous between countries and populations, varying with availability of diagnosis, study design and genetic heterogeneity. In population-based studies, prevalence in males has been estimated to range from 1 in 4,000 and in females from 1 in 8,000. For example, in the United States, the prevalence is approximately 1 in 2,454 males and 1 in 3,717 females. [Crawford D. C. et.al, 2002].

In Europe, the Netherlands has 1 in 6,045 in men and in women 1 in 8000, and the UK has a range of 1 in 2,500 in men and 1 in 4,200 in women because of heterogeneity of studies. Australia has 2.1% intellectually disabled with a prevelance of about 1 in 4,000 men and 1 in 6,000 women. In South Asia, India has a higher prevalence of 1 in 3,600 in men and in 1 in 4,000 in women, possibly because of increased screening in high-risk groups and in East Asia, Japan has 1 in 7,413 in men and 1 in 11,111 in women. [Fazna A & Hagerman R. J. et.al, 2024].

Conversely, Ricaurte is a Colombian town of 1186 inhabitants with the highest prevalence of FXS, which was initially reported by cytogenetic techniques in 1999. On examination, it was noted that out of 1000 inhabitants 48 males and 20 females were fragile X syndrome positive. Around the world, Fragile X syndrome is 343 times more prevalent in males in Ricaurte than at the global level and 226 times more prevalent in females than at the global level. Ricaurte is therefore a unique and central place to investigate the genetics, phenotypes, and potential treatments of Fragile X syndrome. [Saldarriaga W et al, 2018].

In general, overall population prevalence is relatively stable, but special needs populations have significantly greater prevalence, highlighting the need for more genetic testing, early intervention, and region-specific research efforts to more effectively treat and study FXS globally. FXS is inherited in an X-linked dominant pattern.That is, the mutation is on the X chromosome and males (who have one Y and one X chromosome) who have the full mutation will express symptoms of FXS because they don't have a second X chromosome that could potentially override the mutation. Females (who have two X's) but only one of them has the full mutation, may be less severely affected because they have a second, normally functioning, X chromosome. [Bartholomay K.L. et al, 2019].



Conclusion

Fragile X Syndrome (FXS) is the most common inherited cause of intellectual disability, resulting from CGG trinucleotide repeat expansion in the FMR1 gene that leads to gene silencing and loss of FMRP, disrupting normal synaptic and cognitive functions. This review integrates the genetic basis, molecular mechanisms, clinical features, case application, and global epidemiology of FXS, offering a comprehensive perspective that is valuable to genetics, neurology, and clinical research.

The inclusion of a real-world case study bridges laboratory findings with patient-centered outcomes, highlighting innovative management strategies such as CBD-based therapy alongside conventional pharmacological and behavioral interventions. The condition manifests across a broad spectrum of intellectual, behavioral, and physical symptoms, with severity often greater in males due to its X-linked inheritance pattern. Although current management remains largely symptomatic addressing anxiety, behavioral dysregulation, sleep disturbances, and developmental delays early diagnosis, genetic counseling, and timely interventions can markedly improve outcomes. Looking ahead, future research should focus on developing molecular therapies to restore FMR1 function, establishing biomarker-based early detection, conducting long-term studies on emerging treatments, and advancing integrated, personalized care strategies. Broader awareness, global collaboration, and translational research using animal models will be key to discovering novel therapeutic targets and ultimately improving the quality of life for those living with FXS.

Conflict of Interest

There is no conflict of interest related to the study.

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CHAPTER 9: Mercury-Induced Neurotoxicity: Mechanisms, Clinical Manifestations and Therapeutic Considerations

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Abstract

Mercury is a potent environmental neurotoxicant that exists in several chemical forms, each possessing distinct toxicokinetic profiles and mechanisms of neurological injury. This manuscript provides a concise review of mercury-induced neurotoxicity, encompassing the major forms of mercury exposure elemental, inorganic, and organic and their respective pathways of neurotoxic action. The central nervous system (CNS) and peripheral nervous system (PNS) are primary targets, with methylmercury (MeHg) representing the most neurotoxic species due to its high lipophilicity and capacity to cross the blood-brain barrier. Key mechanisms include oxidative stress, mitochondrial dysfunction, disruption of intracellular calcium homeostasis, and impairment of neurotransmitter systems. Clinical manifestations range from subtle cognitive deficits and sensory disturbances to severe motor dysfunction and, in extreme cases, death. Vulnerable populations particularly the developing fetus and young children face disproportionate risk. Current therapeutic strategies, including chelation therapy, are examined alongside their limitations. A deeper understanding of mercury neurotoxicity is essential for informing public health policy, clinical practice, and future research directions.

Keywords: mercury, neurotoxicity, methylmercury, oxidative stress, chelation, blood-brain barrier

Introduction

Mercury (Hg) is one of the most extensively studied environmental neurotoxicants in the history of toxicology. Classified as one of the ten chemicals of major public health concern by the World Health Organization (WHO, 2017), mercury is a naturally occurring heavy metal that is ubiquitously distributed in the environment as a consequence of both geological processes and anthropogenic activities, including coal combustion, artisanal gold mining, and industrial manufacturing. Human exposure occurs through multiple routes, principally through the consumption of mercury-contaminated fish and seafood, inhalation of elemental mercury vapors, and, historically, through occupational exposures (Clarkson & Magos, 2006).

The neurotoxic potential of mercury has been dramatically illustrated by two major historical mass poisoning events: the Minamata disease outbreak in Japan during the 1950s and 1960s, caused by industrial discharge of methylmercury into coastal waters (Harada, 1995), and the

Iraqi grain poisoning incidents of the 1970s, resulting from consumption of methylmercury-treated seed grain (Bakir et al., 1973). These tragedies fundamentally advanced scientific understanding of mercury neurotoxicity and its irreversible consequences. Decades of subsequent research have elucidated the complex molecular, cellular, and systemic mechanisms through which mercury disrupts nervous system function. This manuscript synthesizes current knowledge of mercury-induced neurotoxicity, with emphasis on mechanistic pathways, clinical presentation, and therapeutic interventions.

Forms of Mercury and Routes of Neurotoxic Exposure

Mercury exists in three primary chemical forms: elemental (metallic) mercury (Hg^0), inorganic mercury salts (Hg^{1+} or Hg^{2+}), and organic mercury compounds, of which methylmercury (CH_3Hg^+) is the most toxicologically significant. Each form exhibits distinct physicochemical properties that govern its absorption, distribution, and neurotoxic potential (Clarkson & Magos, 2006).

Elemental mercury, encountered primarily in occupational settings (e.g., dental amalgam preparation, fluorescent lamp manufacturing), volatilizes readily at room temperature. Inhaled mercury vapor is highly lipid-soluble, enabling rapid absorption across alveolar membranes and subsequent oxidation to mercuric ions (Hg^{2+}) within tissues. In the brain, Hg^{2+} accumulates due to the relative inefficiency of efflux mechanisms, producing neurotoxic effects in the cerebral cortex and cerebellum (Rooney, 2014). Chronic low-level inhalation is associated with a characteristic triad of tremor, erethism (a neuropsychiatric syndrome involving irritability, memory loss, and shyness), and gingivitis, historically termed "mad hatter's disease" among felt-hat workers exposed to mercuric nitrate (Waldron, 1983).

Inorganic mercury salts, ingested primarily through contaminated water or certain traditional medicines, are poorly absorbed through the gastrointestinal tract. Nevertheless, systemic absorption sufficient to produce neurotoxic effects can occur, predominantly manifesting as peripheral neuropathy and acrodynia (pink disease) in children (Dinehart et al., 1988). Organic mercury compounds, particularly methylmercury generated by microbial methylation of inorganic mercury in aquatic sediments, represent the dominant neurotoxic threat through dietary exposure. Methylmercury bioaccumulates and biomagnifies through aquatic food chains, reaching highest concentrations in large predatory fish such as tuna, swordfish, and shark (Selin, 2009). Its high lipophilicity and molecular mimicry of amino acids facilitate rapid gastrointestinal absorption (>90%) and efficient transport across the blood-brain barrier (BBB) and placenta via large neutral amino acid carriers, rendering the developing brain exceptionally vulnerable (Clarkson, 2002).

Molecular and Cellular Mechanisms of Neurotoxicity

Mercury exerts neurotoxic effects through multiple convergent mechanisms. Foremost among these is the induction of oxidative stress. Mercury ions exhibit a high affinity for sulfhydryl (-SH) groups, enabling them to inactivate critical antioxidant enzymes—most notably glutathione peroxidase and thioredoxin reductase—and to deplete cellular glutathione (GSH) reserves (Farina et al., 2011). The resulting increase in reactive oxygen species (ROS) initiates lipid peroxidation of neuronal membranes, DNA strand breaks, and protein oxidation, collectively contributing to neuronal dysfunction and apoptosis.

Mitochondrial dysfunction constitutes a second critical mechanism. Mercury disrupts the electron transport chain, particularly at complexes II and III, impairing ATP synthesis and augmenting mitochondrial ROS generation (Dreiem&Seegal, 2007). The bioenergetic failure resulting from mitochondrial compromise is particularly damaging in neurons, which are heavily dependent on oxidative phosphorylation. Disruption of intracellular calcium (Ca^{2+}) homeostasis further amplifies neurotoxicity; mercury increases cytosolic Ca^{2+} concentrations by interfering with Ca^{2+}-ATPase activity and promoting release from intracellular stores, triggering activation of Ca^{2+}-dependent proteases and endonucleases (Limke et al., 2004).

Mercury also disrupts neurotransmitter systems. Inhibition of glutamate transporter function by methylmercury promotes excitotoxic accumulation of extracellular glutamate, overstimulating N-methyl-D-aspartate (NMDA) receptors and initiating excitotoxic cascades (Aschner et al., 2000). Dopaminergic, serotonergic, and cholinergic transmission are also impaired, contributing to the behavioral and cognitive disturbances characteristic of mercury poisoning. Additionally, methylmercury interferes with microtubule polymerization by binding to tubulin, disrupting axonal transport and neuronal migration during development—a mechanism of particular relevance to fetal neurotoxicity (Castoldi et al., 2001).

Clinical Manifestations

The clinical presentation of mercury neurotoxicity varies considerably according to the form of mercury, the route and duration of exposure, and the age and physiological status of the individual. In adults, chronic methylmercury poisoning, as observed in Minamata disease, classically presents with a constellation of sensory disturbances (paresthesias of the distal extremities and perioral region), cerebellar ataxia, dysarthria, constriction of the visual field, and sensorineural hearing loss the Minamata symptom complex (Harada, 1995). Severe cases progress to generalized motor dysfunction, tremor, cognitive decline, and psychiatric disturbances. In the most extreme exposures, coma and death may ensue.

Prenatal methylmercury exposure, even at maternal blood levels insufficient to produce overt maternal symptoms, can result in severe neurodevelopmental consequences in offspring. Fetal Minamata disease, characterized by profound cerebral palsy, intellectual disability, blindness, deafness, and seizures, starkly illustrates the heightened vulnerability of the developing nervous system (Harada, 1995). Epidemiological studies in cohorts with lower-level prenatal exposures—such as the Faroe Islands and Seychelles Island studies—have revealed associations between methylmercury exposure and subtle deficits in attention, memory, language, and fine motor function in children, although the precise threshold for adverse effects remains debated (Grandjean et al., 1997; Davidson et al., 1998).

Elemental mercury vapor exposure produces a characteristic neuropsychiatric syndrome. Early manifestations include insomnia, emotional lability, fatigue, and memory impairment. With continued exposure, intention tremor—initially fine and confined to the hands—may become generalized and disabling. The personality changes constituting erethism, including social withdrawal, excessive timidity, and pathological blushing, are distinctive features

(Rooney, 2014). Peripheral neuropathy, evidenced by diminished tendon reflexes and sensory deficits, is also reported. Recovery following removal from exposure is variable and often incomplete, particularly in cases of prolonged or high-level exposure.

Vulnerable Populations

The developing nervous system is exquisitely sensitive to mercury's neurotoxic effects. During critical windows of neurodevelopment encompassing neuronal proliferation, migration, differentiation, synaptogenesis, and myelination disruption by methylmercury can produce irreversible structural and functional deficits that are disproportionate to the level of exposure that would be required to produce comparable effects in adults (Rice & Barone, 2000). The fetus is exposed transplacentally, and methylmercury concentrations in fetal blood typically exceed maternal concentrations by approximately 20–30%, reflecting active placental transport (Clarkson & Magos, 2006). Breastfeeding infants are also exposed through maternal milk, though at lower levels.

Certain adult populations are at elevated risk due to dietary patterns or occupational exposures. Indigenous and fishing communities that rely heavily on fish and seafood as dietary staples may sustain chronic methylmercury exposures well above reference doses established by regulatory agencies (Selin, 2009). Artisanal and small-scale gold miners, estimated at approximately 10–15 million worldwide, represent a major at-risk group for elemental mercury vapor exposure (WHO, 2017). Genetic polymorphisms affecting mercury toxicokinetics and antioxidant capacity—particularly variants in genes encoding selenoprotein and metallothionein may further modulate individual susceptibility (Custodio et al., 2005).

Therapeutic Approaches and Limitations

The cornerstone of management for mercury neurotoxicity is prompt removal from the source of exposure. For individuals with elevated mercury body burden, chelation therapy employing sulfhydryl-containing compounds principally dimercaptosuccinic acid (DMSA, succimer) and 2,3-dimercapto-1-propanesulfonic acid (DMPS) represents the primary pharmacological intervention. These agents compete with endogenous thiols for mercury binding and enhance urinary mercury excretion (Clarkson et al., 2003). Dimercaprol (BAL), an earlier chelating agent, is now less favored for mercury due to its toxicity profile and lack of superiority over DMSA and DMPS.

Chelation therapy is most effective when initiated early in the course of poisoning, before irreversible neuronal damage is established. Its efficacy in reversing established neurological deficits is limited, as neuronal loss resulting from excitotoxicity and oxidative injury is largely irreparable (Clarkson et al., 2003). Furthermore, conventional chelating agents have limited CNS penetration, restricting their ability to mobilize mercury deposited in deep brain compartments. Experimental approaches aimed at enhancing CNS mercury removal including lipoic acid co-administration and nanoparticle-based delivery systems are under investigation but remain pre-clinical (Pichler et al., 2013). Supportive care, encompassing symptomatic treatment of neurological manifestations (e.g., anticonvulsants for seizures, physical and occupational therapy for motor deficits), constitutes an essential component of management.

Given the irreversibility of established mercury neurotoxicity, primary prevention assumes paramount importance. Regulatory measures limiting mercury emissions, restrictions on mercury use in products and industries, dietary advisories for vulnerable populations regarding fish consumption, and international frameworks such as the Minamata Convention on Mercury (adopted 2013) represent critical public health interventions aimed at reducing global mercury exposure and its neurological consequences (UNEP, 2013).

Conclusion

Mercury-induced neurotoxicity represents a significant and ongoing global public health challenge, arising from the widespread environmental persistence of mercury and the multiplicity of routes through which human exposure occurs. The neurotoxic mechanisms of mercury are multifaceted, involving oxidative stress, mitochondrial impairment, disruption of calcium homeostasis, excitotoxicity, and interference with neurodevelopmental processes. The resulting clinical spectrum ranges from subtle neuropsychological deficits to devastating neurological syndromes, with the developing brain bearing a disproportionate burden of harm. While chelation therapy provides a partial therapeutic option, its limitations underscore the primacy of prevention. Continued research into the molecular underpinnings of mercury neurotoxicity, the identification of genetic and environmental modifiers of susceptibility, and the development of novel neuroprotective and mercury-removal strategies remain critical priorities for protecting neurological health at both individual and population levels.

Conflict of interest

The authors declare no conflict of interest

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CHAPTER 10: Biofilm-Mediated Bioremediation: Recent Advances, Mechanisms and Applications in Industrial Wastewater Treatment

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Abstract

Biofilms-structured, surface-associated microbial communities encased in self-produced extracellular polymeric substances (EPS) have emerged as highly efficient biological systems for the remediation of industrial wastewater. Unlike planktonic microbial cultures, biofilms offer enhanced metabolic diversity, superior resistance to toxic compounds, and sustained activity under fluctuating environmental conditions. Recent advances in microbiology, molecular ecology, and bioprocess engineering have accelerated our understanding of biofilm architecture, quorum sensing (QS)-regulated community behaviour, and the roles of diverse microbial taxa in pollutant degradation. This chapter provides a comprehensive review of the recent literature on biofilm-mediated bioremediation, encompassing the structural and functional basis of biofilms, mechanisms of pollutant removal including biosorption, biotransformation, and biomineralization, the role of quorum sensing and the EPS matrix in remediation efficiency, emerging engineered biofilm systems such as moving bed biofilm reactors (MBBRs) and membrane biofilm reactors (MBfRs), and the integration of omics technologies for biofilm community profiling. Applications across a spectrum of industrial effluents including textile, tannery, pharmaceutical, and heavy metal-laden wastewaters are critically evaluated. Limitations, regulatory considerations, and future directions encompassing synthetic biology and nanotechnology-enhanced biofilm systems are also addressed.

Keywords: biofilm, bioremediation, extracellular polymeric substances, quorum sensing, industrial wastewater, heavy metals, moving bed biofilm reactor, omics

1. Introduction

Water pollution arising from rapid industrialization, urbanization, and agricultural intensification remains one of the most pressing environmental challenges of the twenty-first century. Industrial effluents released from textile, tannery, pharmaceutical, petrochemical, and electroplating sectors carry complex cocktails of heavy metals, synthetic dyes, endocrine-disrupting compounds, and recalcitrant organic molecules that threaten aquatic ecosystems and public health (Dutta et al., 2021; Ojha et al., 2021). Conventional physicochemical treatment technologies including chemical precipitation, membrane filtration, ion exchange, and electrochemical oxidation although effective for certain pollutant classes, are frequently criticized for their high operational costs, secondary sludge generation, and inability to cope with the heterogeneous nature of real industrial effluents (Saeed et al., 2022).

Bioremediation, broadly defined as the use of living organisms or their metabolic products to detoxify or transform environmental contaminants, has gained significant traction as a green, cost-effective, and scalable alternative (Luka & Highina, 2018; Abatenh et al., 2017). Within the domain of microbial bioremediation, the significance of biofilms has been increasingly recognized over the last decade. Biofilms are structured consortia of microorganisms attached to solid surfaces and embedded within a self-secreted matrix of extracellular polymeric substances (EPS) comprising polysaccharides, proteins, nucleic acids, and lipids (Flemming & Wingender, 2010). This sessile mode of life confers on biofilm-dwelling microorganisms extraordinary tolerance to environmental stresses, antibiotics, and heavy metal toxicity — properties that make them particularly well suited to the harsh conditions encountered in industrial wastewater streams.

The shift from single-organism, planktonic-based remediation strategies to multispecies biofilm systems reflects a growing appreciation that microbial communities in nature operate as cooperative networks, deploying complementary metabolic pathways to mineralize complex pollutants that no single species could degrade alone (Singh et al., 2022). Biofilm reactors have consequently become central to next-generation biological treatment systems, with engineered configurations such as moving bed biofilm reactors (MBBRs), rotating biological contactors (RBCs), and membrane biofilm reactors (MBfRs) now deployed at industrial scale worldwide (Ødegaard, 2006; Martin & Nerenberg, 2012).

Recent years have also witnessed the application of high-throughput sequencing, metagenomics, metatranscriptomics, and metaproteomics to biofilm communities, enabling unprecedented resolution of community composition, functional gene repertoires, and active metabolic pathways under remediation conditions (Mande et al., 2012; Tyson et al., 2004). Simultaneously, synthetic biology approaches including the design of quorum-sensing circuits, surface-adhesion modules, and pathway engineering are opening new frontiers in the rational design of remediation biofilms with expanded substrate ranges and enhanced pollutant transformation rates (Brenner et al., 2008).

This chapter synthesizes the rapidly evolving literature on biofilm-mediated bioremediation, focusing specifically on mechanistic advances made since 2015. It examines the structural biology of biofilms, the molecular mechanisms underpinning pollutant removal, the performance of engineered biofilm reactors, the application of omics tools to community analysis, and the promising role of emerging technologies in overcoming existing limitations. The goal is to provide researchers, environmental engineers, and policymakers with a state-of-the-art reference that bridges fundamental microbiology with applied wastewater treatment science.

2. Biofilm Architecture and the Extracellular Polymeric Substance Matrix

2.1 Stages of Biofilm Development

Biofilm formation is a highly regulated, multi-stage process. The canonical developmental sequence comprises: (i) reversible attachment of planktonic cells to a conditioned surface, mediated by non-specific van der Waals and electrostatic forces; (ii) irreversible attachment facilitated by type IV pili, flagella, and surface adhesins; (iii) microcolony formation and initial EPS synthesis; (iv) biofilm maturation characterized by three-dimensional architecture with water channels enabling nutrient and oxygen transport; and (v) dispersal, during which cells revert to the planktonic state and colonize new surfaces (Stoodley et al., 2002; Hall-Stoodley et al., 2004). Each stage is regulated by complex genetic circuits, environmental signals including nutrient availability, hydrodynamic shear, and quorum sensing molecules (Karatan & Watnick, 2009).

2.2 EPS Composition and Function

The EPS matrix, which can constitute up to 90% of the biofilm dry mass, is far more than a passive scaffold. Flemming et al. (2016) described EPS as the 'house of the biofilm cell,' providing mechanical stability, mediating surface adhesion, enabling intercellular communication, and functioning as an external digestive system by retaining hydrolytic enzymes in close proximity to cells. The EPS also plays a central role in bioremediation through the sorption of heavy metals and organic pollutants onto negatively charged polysaccharide and protein residues, a process now referred to as EPS-mediated biosorption (More et al., 2014). Recent research has demonstrated that the composition of EPS is highly plastic and responsive to environmental stressors: exposure to cadmium, chromium, or zinc ions induces upregulation of EPS-biosynthetic genes and results in altered polymer compositions with higher metal-binding capacity (Gupta & Diwan, 2017).

2.3 Spatial Organization and Metabolic Gradients

A defining feature of mature biofilms is their spatial heterogeneity. Oxygen, nutrient, pH, and redox gradients created by diffusion limitation and metabolic consumption establish distinct microniches within the biofilm cross-section. Aerobic metabolism dominates the surface layers, while anaerobic processes — fermentation, sulfate reduction, methanogenesis — operate in the deeper, anoxic zones (Rittmann & McCarty, 2001). This spatial organization means that a single biofilm can simultaneously carry out aerobic nitrification near its surface and anaerobic denitrification at its base, a phenomenon exploited in simultaneous nitrification-denitrification (SND) reactor designs (Gao et al., 2011). Similarly, in heavy metal-contaminated systems, aerobic oxidation and anaerobic sulfate reduction can occur concurrently, enabling both enzymatic transformation and precipitation of metal sulfides within the same biofilm (White & Gadd, 1998).

3. Quorum Sensing and Cooperative Behaviour in Remediation Biofilms

Quorum sensing (QS) is the cell density-dependent intercellular signalling system through which bacteria coordinate gene expression at the community level. In Gram-negative bacteria, N-acyl homoserine lactones (AHLs) serve as the predominant QS signals, while Gram-positive bacteria typically use oligopeptide autoinducers; autoinducer-2 (AI-2) mediates interspecies communication across both domains (Ng & Bassler, 2009). In the context of biofilm formation and bioremediation, QS regulates a range of functions directly relevant to pollutant removal, including EPS biosynthesis, biofilm maturation, production of biosurfactants, secretion of extracellular enzymes, and induction of stress-response pathways (Huang et al., 2016).

Recent studies have demonstrated that QS-deficient mutants form structurally aberrant biofilms with reduced EPS production and consequently impaired metal biosorption capacity. Pseudomonas aeruginosa lasI/rhlI double mutants, for instance, formed flat, undifferentiated biofilms with markedly reduced capacity to sequester cadmium compared to wild-type strains (Huang et al., 2016). Conversely, exogenous supplementation of synthetic AHL analogues has been shown to enhance biofilm formation in mixed-community wastewater biofilms, suggesting a potential strategy for boosting remediation performance (Li & Tian, 2012). The discovery that many environmental bacteria produce QS-quenching enzymes (acylases and lactonases) capable of degrading AHL signals has raised important questions about the stability of QS-driven cooperation in complex wastewater environments, and has also opened a new avenue for biofilm engineering through deliberate manipulation of the QS network (Dong et al., 2007).

Quorum sensing also governs biofilm dispersal, the final stage of the biofilm life cycle. Dispersal is triggered by signals including nitric oxide (NO), cis-2-decenoic acid, and the secondary messenger c-di-GMP, whose intracellular concentration drops sharply at dispersal induction (Römling et al., 2013). In reactor systems, controlled induction of dispersal could be used to slough off metal-laden or pollutant-saturated biofilm biomass, enabling periodic regeneration of active biofilm surfaces — a strategy with considerable practical potential for biofilm reactor management.

4. Mechanisms of Pollutant Removal by Biofilms

4.1 Biosorption

Biosorption refers to the passive, metabolism-independent binding of pollutants — particularly heavy metal cations — to functional groups on microbial cell surfaces and EPS components. Carboxyl, hydroxyl, phosphoryl, amino, and sulfhydryl groups on polysaccharides, proteins, and teichoic acids carry net negative charges at environmental pH values and provide abundant metal-binding sites (Wang & Chen, 2009). Biosorption is rapid, reversible, and applicable to both living and dead biomass, making it an attractive component of biofilm-based treatment systems. The biosorption capacity of biofilm EPS for heavy metals such as Pb^{2+}, Cd^{2+}, Cu^{2+}, and Cr^{6+} significantly exceeds that of equivalent planktonic biomass, due to the higher surface-to-volume ratio and the concentration of EPS-binding sites (More et al., 2014).

Isotherm modelling using Langmuir and Freundlich equations has been widely applied to quantify biosorption equilibria, while kinetic studies employing pseudo-first- and pseudo-second-order models describe the time course of metal uptake. Recent work has moved beyond bulk isotherm characterisation to probe biosorption mechanisms at the molecular level using extended X-ray absorption fine structure (EXAFS) spectroscopy, confirming that metals coordinate predominantly with carboxylate and phosphate groups in EPS polysaccharides (Gupta & Diwan, 2017).

4.2 Biotransformation and Enzymatic Degradation

Biofilm microorganisms enzymatically transform organic pollutants through oxidation, reduction, hydrolysis, dehalogenation, and ring-cleavage reactions. Ligninolytic enzymes produced by white-rot fungi — including laccase, manganese peroxidase, and lignin peroxidase — degrade polycyclic aromatic hydrocarbons (PAHs), synthetic dyes, and polychlorinated biphenyls (PCBs) through radical-mediated oxidation (Pointing, 2001). In bacterial biofilms, dioxygenases, monooxygenases, and reductive dehalogenases catalyse the initial activation of aromatic rings and aliphatic chlorinated compounds, with downstream β-ketoadipate and modified ortho-cleavage pathways channelling intermediates into central carbon metabolism (Diaz, 2004).

An important recent development is the recognition that EPS-associated extracellular enzymes extend the catalytic reach of biofilms beyond the cellular interior. Extracellular laccases, peroxidases, and proteases retained within the EPS matrix continue to catalyse pollutant transformations even in the absence of direct cell contact, effectively expanding the reactive volume of the biofilm (Flemming et al., 2016). In lignin-rich paper and pulp effluents, extracellular peroxidase activity associated with Phanerochaete chrysosporium and Trametes versicolor biofilms has been shown to decolourise recalcitrant effluents more efficiently than equivalent suspended-cell systems, owing to the protective effect of EPS against oxidative inactivation of enzymes (Pointing, 2001).

4.3 Biomineralization and Metal Precipitation

In addition to biosorption, biofilm communities can precipitate dissolved metals as insoluble sulfide, carbonate, phosphate, or hydroxide minerals through metabolically driven changes in local geochemistry. Sulfate-reducing bacteria (SRB) within anaerobic biofilm zones produce H_2S, which reacts with dissolved heavy metals to form highly insoluble metal sulfide precipitates (ZnS, PbS, CdS, CuS), effectively removing them from solution (White & Gadd, 1998). This process, termed biomineralization or biogenic metal sulfide precipitation, has been harnessed in passive bioreactor systems for treatment of acid mine drainage, achieving greater than 99% removal of Zn, Pb, and Cd at low operational cost (Nancucheo & Johnson, 2012).

More recently, phosphate-mineralizing biofilms have been investigated for the removal of uranium and rare earth elements through biogenic formation of metal-phosphate precipitates, a mechanism relevant to nuclear waste management and critical metal recovery (Merroun & Selenska-Pobell, 2008). The spatial coupling of SRB-dominated zones with aerobic iron-oxidizing bacteria at the biofilm-solution interface has been described in circumneutral pH systems, where aerobic iron oxidation generates ferric hydroxide flocs that co-precipitate additional metals — a coupled biogeochemical mechanism of particular significance in constructed wetland biofilm systems.

4.4 Bioaccumulation and Intracellular Sequestration

Unlike biosorption, bioaccumulation is an active, energy-dependent process in which metal ions are transported across the cell membrane and sequestered intracellularly by metallothioneins, polyphosphate bodies, or vacuoles. In biofilms, the EPS matrix acts as a pre-concentrating layer that increases the local metal concentration at the cell surface, facilitating subsequent intracellular uptake (Gadd, 2010). Genetic studies have demonstrated that biofilm cells upregulate metal-efflux pumps (e.g., CzcABC in Cupriavidus metallidurans) and metallothionein-encoding genes (e.g., smtA in cyanobacteria) in response to metal challenge, reducing intracellular metal toxicity while simultaneously contributing to metal removal from the external medium (Nies, 2003).

5. Engineered Biofilm Reactor Systems for Industrial Wastewater Treatment

5.1 Moving Bed Biofilm Reactors (MBBRs)

The moving bed biofilm reactor (MBBR), developed in Norway in the late 1980s, uses freely floating plastic carrier media to support biofilm growth while the bulk liquid phase remains well-mixed. The high specific surface area of modern carriers (500–1200 m^2/m^3) provides extensive biofilm colonization area, while continuous movement prevents carrier clogging and maintains biofilm thickness at optimal levels through shear-controlled sloughing (Ødegaard, 2006). MBBRs have demonstrated impressive performance across a range of industrial wastewaters: nitrogen removal efficiencies exceeding 90% have been documented in pharmaceutical effluent treatment, while combined MBBRs treating textile wastewater achieved simultaneous colour removal (>85%) and COD reduction (>80%) under aerobic-anoxic operating conditions (Hai et al., 2010).

A significant recent advance is the integration of MBBR technology with membrane bioreactors (MBR), creating hybrid MBBR-MBR systems that combine the biodegradation efficiency of biofilm with the solid-liquid separation performance of ultrafiltration membranes. These systems reduce membrane fouling — a perennial challenge in conventional MBRs — because the carrier media absorb peak organic and hydraulic loads that would otherwise impact membrane performance (Yang et al., 2009).

5.2 Membrane Biofilm Reactors (MBfRs)

In membrane biofilm reactors (MBfRs), biofilm grows on the outside surface of hollow-fibre membranes through which a gas (O_2, H_2, or CH_4) is delivered without bubble formation (Martin & Nerenberg, 2012). This configuration enables precise delivery of electron acceptors or donors to the biofilm, independent of bulk liquid concentrations, and allows simultaneous removal of pollutants requiring different redox conditions. Oxygen-based MBfRs have been applied to aerobic treatment of high-strength industrial wastewaters, while hydrogen-based MBfRs have been used for perchlorate, nitrate, and selenate bioreduction in groundwater and mining effluents. Recent pilot-scale demonstrations of MBfRs treating chromate-containing electroplating wastewater achieved Cr(VI) reduction below detection limits using a hydrogen-fed, mixed SRB-denitrifying biofilm (Rittmann et al., 2004).

5.3 Rotating Biological Contactors (RBCs) and Trickling Filters

Rotating biological contactors consist of large-diameter discs partially submerged in wastewater and rotating to alternately expose the biofilm to wastewater and air, enabling oxygen transfer and pollutant contact. Recent modifications incorporate mixed-community biofilms including cyanobacteria, which contribute both photosynthetically derived oxygen and direct phototrophic pollutant uptake, reducing aeration costs in the treatment of nutrient-rich agro-industrial effluents (Craggs et al., 2012). Trickling filters, one of the oldest biofilm technologies, have experienced renewed interest through the introduction of structured plastic media with improved hydraulic characteristics and the inoculation of specialized microbial consortia capable of degrading emerging contaminants such as pharmaceutical residues and personal care products (Suarez et al., 2010).

5.4 Granular Aerobic and Anaerobic Sludge Systems

Aerobic granular sludge (AGS) represents a self-immobilized biofilm variant in which microbial granules — compact, spherical bioaggregates — form through hydrodynamic selection in sequencing batch reactors operated under specific feast-famine conditions (Liu & Tay, 2002). Aerobic granules exhibit simultaneous nitrification-denitrification and biological phosphorus removal without external carbon addition, and their compact structure and excellent settling velocity offer significant advantages over conventional activated sludge for high-strength industrial wastewater treatment. Recent full-scale AGS installations at breweries and food-processing facilities have demonstrated 85–95% COD removal and complete nitrogen removal at hydraulic retention times of 4–6 hours, with a biomass footprint 75% smaller than equivalent conventional systems (Pronk et al., 2015). In the anaerobic domain, upflow anaerobic sludge blanket (UASB) reactors exploit the formation of dense granular biofilms to treat high-COD industrial effluents such as distillery stillage, pharmaceutical waste, and dairy effluent, achieving COD removal efficiencies of 85–98% while generating methane as a recoverable energy product (van Lier et al., 2015).

6. Recent Applications of Biofilm-Mediated Bioremediation Across Industrial Sectors

6.1 Textile and Dye Wastewater

Textile wastewater, characterized by intense colour, high salinity, alkaline pH, and refractory azo dye content, represents a particularly challenging matrix for biological treatment (Saeed et al., 2022). Azo dyes, which account for approximately 70% of synthetic dyes used globally, possess N=N chromophoric groups resistant to aerobic degradation but susceptible to anaerobic reductive cleavage, yielding colourless but potentially carcinogenic aromatic amine intermediates that must be subsequently mineralized aerobically. Biofilm systems configured with sequential anaerobic-aerobic zones have emerged as the preferred approach, enabling complete decolourisation and mineralization of aromatic amines in a single reactor train (Hai et al., 2010).

Notable recent contributions include the development of Pseudomonas-Bacillus co-culture biofilms that decolourise Reactive Black 5 and Direct Red 23 at rates exceeding those of monoculture biofilms by 2–3-fold, attributed to complementary enzyme profiles (azoreductase and laccase) and QS-mediated metabolic cooperation (Jin et al., 2012). Microalgal-bacterial biofilms grown in photobioreactors have demonstrated simultaneous colour removal and CO_2 fixation when treating textile effluents, providing a carbon-neutral treatment pathway for small-scale textile producers (Craggs et al., 2012).

6.2 Tannery and Chromium-Containing Wastewater

Chromium hexavalent [Cr(VI)], widely used in tannery, electroplating, and metal-finishing industries, is a potent carcinogen and mutagen that must be reduced to the far less toxic trivalent form [Cr(III)] prior to disposal (Banik et al., 2023). A diverse array of biofilm-forming bacteria, including Pseudomonas putida, Bacillus cereus, Lysinibacillus sphaericus, and Ochrobactrum intermedium, mediate Cr(VI) reduction via membrane-associated chromate reductases and soluble NADH-dependent enzymes (Viti et al., 2014). In biofilm configurations, these organisms achieve complete Cr(VI) reduction at concentrations up to 200 mg/L, values that would be lethal to planktonic equivalents, underscoring the protective effect of EPS.

Sulfate-reducing bacteria biofilms have been particularly effective in combined Cr(VI) and sulfate removal from tannery effluents, as biogenic H_2S both reduces Cr(VI) and precipitates Cr(III) as insoluble chromium sulfide. Recent metagenomics studies of tannery treatment biofilms have revealed unexpectedly diverse Cr(VI)-reducing communities, including archaea and uncultured lineages, suggesting that the full bioremediation potential of such communities remains to be characterized (Viti et al., 2014).

6.3 Pharmaceutical and Emerging Contaminant Removal

Pharmaceutical compounds and personal care products (PPCPs) — including antibiotics, anti-inflammatory drugs, steroid hormones, and UV filters — are detected ubiquitously in effluents from pharmaceutical manufacturing plants and municipal wastewater treatment plants and are recognised as emerging contaminants of significant ecological concern (Suarez et al., 2010). Conventional activated sludge systems often achieve only 20–60% removal of PPCPs, whereas biofilm-based systems — particularly those with long biomass retention times and diverse microbial communities — achieve substantially higher removal efficiencies. Nitrifying biofilms have been identified as critical for the cometabolic oxidation of estrogens and pharmaceuticals via ammonia monooxygenase, an enzyme with broad substrate specificity (Suarez et al., 2010).

The removal of antibiotics by biofilm systems is complicated by the simultaneous selection of antibiotic-resistant bacteria and the transfer of antibiotic resistance genes (ARGs) via horizontal gene transfer within the biofilm. This represents a significant emerging concern at the interface of bioremediation and public health, and has stimulated research into biofilm-based systems that remove antibiotics while minimizing ARG dissemination, for example through integration with UV disinfection or advanced oxidation processes (Pruden et al., 2013).

6.4 Heavy Metal Removal from Mining and Metallurgical Effluents

Acid mine drainage (AMD), generated by the oxidative dissolution of pyrite and other sulfidic minerals exposed during mining operations, is characterized by extremely low pH (often < 3), high dissolved sulfate, and elevated concentrations of Fe, As, Zn, Pb, Ni, and other metals. Passive treatment of AMD using constructed wetlands and permeable reactive barriers colonized by diverse sulfate-reducing and iron-reducing biofilms has achieved long-term metal removal at low operational cost (Nancucheo & Johnson, 2012). Biofilm communities in AMD passive treatment systems are dominated by acidophilic SRB (Desulfosporosinus spp.) and fermentative bacteria that sustain SRB activity by providing electron donors from organic matter degradation.

Innovative bioelectrochemical systems (BES), including microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) with biofilm-coated electrodes, are emerging as dual-function platforms that simultaneously treat metal-laden effluents and generate electricity or hydrogen. In MFC configurations treating AMD, biofilm anodes oxidize organic carbon while cathode-associated communities reduce Fe^{3+} and Cr(VI), achieving simultaneous energy recovery and metal detoxification (Borole et al., 2011).

7. Application of Omics Technologies to Biofilm Community Characterization

The advent of high-throughput sequencing and allied omics technologies has transformed our ability to characterize the structure and function of complex biofilm communities in industrial wastewater systems. 16S rRNA gene amplicon sequencing provides rapid, culture-independent snapshots of community composition across space and time, revealing the succession of microbial taxa from initial colonisers through mature remediation communities (Mande et al., 2012). Metagenomic analysis of shotgun-sequenced biofilm DNA provides access to the functional gene repertoire of the community — including genes encoding key catabolic enzymes, metal resistance determinants, and EPS biosynthetic pathways — without reliance on culturability (Tyson et al., 2004).

Metatranscriptomics — the sequencing of community mRNA — resolves which genes are actively transcribed under specific remediation conditions, distinguishing metabolically active populations from dormant members. Recent metatranscriptomic studies of MBBR biofilms treating pharmaceutical effluent identified a core set of 47 actively expressed catabolic genes responsible for >90% of measured PPCP removal, providing the first molecular-level account of pharmaceutical bioremediation activity in a complex biofilm (Pruden et al., 2013). Metaproteomics extends this resolution to the protein level, enabling direct identification of expressed enzymes involved in pollutant transformation and EPS biosynthesis (Mande et al., 2012).

Integrative multi-omics approaches that combine metagenomics, metatranscriptomics, and metabolomics are now being applied to construct genome-scale metabolic models of biofilm communities, enabling in silico prediction of community behaviour under varying substrate and pollutant conditions. Such models have successfully predicted the performance of nitrifying-denitrifying biofilms under transient nitrogen loads, and are beginning to be applied to the more complex challenge of modelling mixed-function remediation communities treating multi-contaminant industrial effluents (Borole et al., 2011).

8. Synthetic Biology and Nanotechnology Approaches to Biofilm Engineering

Synthetic biology offers powerful tools for the rational engineering of biofilm communities with enhanced remediation capabilities. Gene circuits designed to upregulate EPS biosynthesis, metal-chelating peptide expression, or specific catabolic pathways in response to pollutant concentrations have been introduced into model biofilm-forming organisms including Pseudomonas putida and Bacillus subtilis (Brenner et al., 2008). Kill switches and containment strategies — including toxin-antitoxin systems and synthetic auxotrophies — have been incorporated to prevent environmental release of engineered strains, addressing biosafety concerns.

The integration of nanotechnology with biofilm systems represents a particularly fertile area of recent research. Nano zero-valent iron (nZVI) incorporated into biofilm EPS enhances reductive dechlorination of chlorinated solvents through combined abiotic and biotic mechanisms, with the biofilm providing a protective microenvironment that prevents nZVI oxidation and aggregation (Su et al., 2012). Magnetic nanoparticles functionalized with metal-binding peptides and embedded in biofilm matrices enable magnetic separation of metal-laden biomass, facilitating both metal recovery and biomass regeneration. Carbon nanotube- and graphene oxide-amended biofilm carriers have been shown to enhance electron transfer rates between bacterial cells and electrodes in BES applications, improving current generation and metal reduction efficiency (Borole et al., 2011).

Biofilm engineering through the programming of spatial consortium structure — for instance, positioning specialist nitrifiers, denitrifiers, and metal-reducers in defined spatial zones using layer-by-layer deposition on structured carriers — represents an ambitious but increasingly achievable goal. Microfluidic platforms that allow real-time imaging and manipulation of biofilm community structure at micron resolution are facilitating the design principles needed for such precision biofilm engineering (Rittmann & McCarty, 2001).

9. Limitations and Challenges

Despite considerable advances, biofilm-mediated bioremediation confronts a number of significant limitations. Biofilm formation is inherently sensitive to hydrodynamic conditions, surface chemistry, and the composition of the incoming wastewater, making it difficult to maintain consistent biofilm thickness and activity under the variable conditions typical of real industrial effluents. Biofilm clogging in fixed-bed systems leads to channelling, dead zones, and hydraulic short-circuiting, reducing treatment efficiency and necessitating periodic backwashing or media replacement (Ødegaard, 2006).

The production of hazardous intermediate metabolites during incomplete degradation of complex organic pollutants — as exemplified by the accumulation of carcinogenic aromatic amines during incomplete azo dye reduction — necessitates careful system design and monitoring to ensure that biofilm treatment does not merely transform one class of hazardous compound into another. The scale-up of laboratory-scale biofilm systems to pilot and full industrial scale frequently encounters unanticipated challenges related to oxygen transfer limitation, biofilm heterogeneity, and the emergence of non-target community members that outcompete the desired degraders (Saeed et al., 2022).

Regulatory frameworks governing the deliberate release of genetically modified microorganisms in open biofilm systems remain stringent in most jurisdictions, limiting the near-term deployment of engineered biofilm strains in full-scale treatment. The long-term stability of both natural and engineered biofilm communities under varying environmental conditions, and particularly under the selection pressure of complex industrial effluents containing antibiotics, biocides, and heavy metals, remains a critical unanswered question requiring longitudinal ecological studies.

10. Future Prospects

The trajectory of biofilm bioremediation research points towards several high-impact directions. The integration of machine learning and artificial intelligence with real-time biofilm monitoring data — including online sensors for dissolved oxygen, redox potential, effluent quality, and biofilm thickness — promises to enable adaptive, model-predictive control of biofilm reactor systems that maximize pollutant removal under dynamic influent conditions (Borole et al., 2011). The emerging field of electroactive biofilm research, which examines the capacity of Geobacter, Shewanella, and affiliated taxa to transfer electrons extracellularly via conductive pili ('bacterial nanowires') and soluble electron shuttles, opens new avenues for the reductive removal of redox-active pollutants including Cr(VI), U(VI), and nitrate in bioelectrochemical systems with minimal chemical input.

Advances in synthetic biology, including CRISPR-Cas9 genome editing, cell-free expression systems, and the design of programmable genetic circuits, will accelerate the creation of chassis organisms with expanded substrate ranges, enhanced metal tolerance, and tightly regulated biosafety features. The coupling of biofilm remediation with resource recovery — extracting valuable metals, nitrogen, phosphorus, and biogas from wastewaters — represents a paradigm shift from treatment as a cost centre to treatment as a value-generating process, aligned with the circular economy principles increasingly adopted by advanced industrial nations (van Lier et al., 2015). International research networks and standardized reporting frameworks will be essential to accelerate the transition of promising laboratory-scale biofilm technologies to full industrial deployment.

11. Conclusion

Biofilm-mediated bioremediation represents a scientifically mature yet rapidly evolving field that offers compelling advantages over conventional physicochemical wastewater treatment technologies. The structural complexity of biofilms — their EPS architecture, quorum-sensing regulated cooperation, and spatially organized metabolic gradients — underpins their exceptional capacity to degrade, transform, and immobilize a diverse array of industrial pollutants under conditions that would be inhibitory to equivalent planktonic systems. Engineered biofilm reactor configurations, including MBBRs, MBfRs, and aerobic granular sludge systems, have demonstrated robust full-scale performance across textile, tannery, pharmaceutical, and mining wastewater applications. The application of omics technologies continues to reveal the extraordinary functional diversity of biofilm communities and to identify novel degradative pathways with biotechnological potential. Synthetic biology and nanotechnology are providing new tools to design next-generation remediation biofilms with programmable function and enhanced performance. Realizing the full potential of biofilm bioremediation will require sustained interdisciplinary collaboration across microbiology, environmental engineering, systems biology, materials science, and regulatory science, as well as continued investment in pilot-scale validation and long-term performance monitoring under real industrial conditions.

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CHAPTER 11: Endospore-Forming Bacteria as Next-Generation Biofertilizers Under Abiotic Stress Conditions: Mechanisms, Applications and Future Perspectives

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Abstract

Soil-dwelling endospore-forming bacteria represent a uniquely durable class of microorganisms whose capacity to survive extreme environmental conditions makes them exceptional candidates for next-generation biofertilizer development. As global agriculture faces mounting pressures from climate change, soil degradation, and the imperative to reduce synthetic chemical inputs, these bacteria, particularly members of the genera Bacillus, Paenibacillus, Clostridium, and Frankia; offer a biologically sustainable solution to enhance crop productivity under abiotic stress. This review comprehensively synthesizes current knowledge on the taxonomy, ecology, and stress-tolerance mechanisms of endospore-forming plant growth-promoting rhizobacteria (PGPR), with emphasis on their roles in nitrogen fixation, phosphate solubilization, phytohormone production, and biocontrol under conditions of drought, salinity, extreme temperature, and elevated pH. Drawing on evidence from over five decades of applied microbiology and agronomy, we analyze field-scale and greenhouse case studies demonstrating the efficacy of endospore-based biofertilizers in diverse agro-climatic contexts. We also explore recent mechanistic insights into sporulation-induced gene expression, dormancy-revival cycles, and exo-polysaccharide-mediated soil aggregation, which collectively underpin the agronomic value of these organisms. Critical gaps, including limited understanding of community-level interactions, inconsistent field performance, and regulatory barriers are identified alongside promising future directions such as consortium-based inoculants, omics-guided strain improvement, and nano-encapsulation delivery systems. This review underscores the central importance of endospore-forming bacteria in the transition toward climate-resilient, chemically reduced agriculture.

Keywords: Endospore-forming bacteria, Bacillus spp., Paenibacillus spp., biofertilizers, plant growth-promoting rhizobacteria (PGPR), abiotic stress tolerance, nitrogen fixation, phosphate solubilization, sustainable agriculture, sporulation.

1. Introduction

Agriculture stands at a critical crossroads. The global population, projected to exceed 9.7 billion by 2050, demands a 60–70% increase in food production, yet conventional approaches relying on synthetic nitrogen and phosphate fertilizers are increasingly untenable. These inputs are energetically costly, environmentally harmful, and subject to rapidly depleting finite resources. In parallel, climate change is intensifying the frequency and severity of abiotic stresses such as drought, salinity, temperature extremes, and heavy metal contamination - that impose substantial yield penalties across staple crops worldwide. The imperative to develop biologically grounded, ecologically sustainable alternatives has never been more urgent.

Among the diverse microbial communities that colonize the rhizosphere and bulk soil, endospore-forming bacteria occupy a particularly privileged ecological niche. Their defining characteristic - the capacity to form highly resistant, metabolically dormant endospores in response to environmental adversityconfers upon them an unparalleled resilience that most vegetative bacteria lack. Endospores are arguably the most durable biological structures known: they can withstand temperatures exceeding 120°C, ultraviolet radiation, chemical disinfectants, desiccation for centuries, and nutrient starvation, yet remain capable of rapid germination and metabolic revival upon the return of favourable conditions.

The agronomic potential of these organisms was recognized as early as the 1950s, when Bacillus-based preparations were first explored as soil inoculants. However, the systematic study of their plant growth-promoting (PGP) capabilities - encompassing biological nitrogen fixation, phosphate solubilization, phytohormone biosynthesis, siderophore production, volatile organic compound emission, and biocontrol of soilborne pathogens has accelerated dramatically over the past two decades. Landmark genomic studies of strains such as *Bacillus amyloliquefaciens* FZB42 and Bacillus subtilis 168 have revealed the genetic blueprints underlying these diverse functional traits, while field trials across South Asia, Sub-Saharan Africa, and Latin America have documented meaningful yield improvements under stress conditions.

This review synthesizes the current state of knowledge on endospore-forming bacteria as biofertilizers, with particular emphasis on their performance under abiotic stress. We examine the taxonomic diversity and ecophysiology of these organisms, dissect the molecular and biochemical mechanisms by which they promote plant growth, review the evidence from application studies conducted under controlled and field conditions, and critically evaluate the limitations and opportunities that define this rapidly evolving field. Our goal is to provide a rigorous, integrative perspective that serves both the academic researcher and the agronomic practitioner navigating the path toward sustainable food production.

2. Literature Review

2.1 Taxonomy and Diversity of Endospore-Forming Bacteria

Endospore formation is a phylogenetically conserved trait found predominantly within the phylum Firmicutes, though it also occurs in certain members of Actinobacteria (notably Frankia spp.) and Cyanobacteria. Within Firmicutes, the order Bacillales - encompassing the families Bacillaceae, Paenibacillaceae, Sporolactobacillaceae, and others, contains the vast majority of agriculturally relevant endospore-formers. The genus Bacillus alone comprises over 200 validly described species, of which a substantial subset has demonstrated plant growth-promoting activity.

2.1.1 Key Taxa and Their Agronomic Strains

Table 1 summarizes the principal endospore-forming bacterial taxa investigated as biofertilizers, along with notable strains, stress tolerances, and primary functional roles.

Table 1. Principal Endospore-Forming Bacterial Species Investigated as Biofertilizers

Species	Notable Strains	Key Stress Tolerance	Primary Biofertilization Role
Bacillus subtilis	NRRL B-30408, FZB42, SL-13	Drought, heat, salinity	IAA production, phosphate solubilization, PGPR
Bacillus amyloliquefaciens	FZB42, GB03, IN937a	Salinity, extreme pH	Nitrogen fixation, antifungal compounds
Bacillus licheniformis	ATCC 14580, K11	Heat, UV radiation	Phosphate solubilization, siderophore production
Bacillus megaterium	WH1, NBRI2400	Drought, salinity	Phosphate solubilization, IAA, cytokinin
Bacillus pumilus	SAFR-032, HR10	UV radiation, desiccation	Induced systemic resistance, ACC deaminase
Paenibacillus polymyxa	E681, B2, SC36	Low temperature, drought	Nitrogen fixation, cytokinin, exopolysaccharides
Clostridium pasteurianum	W5, ATCC 6013	Anaerobic, waterlogged soils	Biological nitrogen fixation
Lysinibacillus sphaericus	OT4b.31, CBAM5	Heavy metal contamination	Metal bioremediation, IAA
Frankia spp.	ACN1, CcI3	Arid, nutrient-poor soils	Actinorhizal nitrogen fixation

FC = Field Capacity; IAA = Indole-3-acetic acid; PGPR = Plant Growth-Promoting Rhizobacteria

Bacillus subtilis and *B. amyloliquefaciens* are the most extensively studied species, with sequenced type strains (FZB42, 168, NRRL B-30408) serving as model systems for understanding PGP gene clusters, including those encoding non-ribosomal peptide synthetases (NRPS) for **iturin**, **fengycin**, and **surfactin** biosynthesis. *Paenibacillus polymyxa*, formerly classified as *B. polymyxa*, is distinguished by its nitrogenase gene complex (nif operon) enabling atmospheric nitrogen fixation - a trait less common among aerobic Bacillus species. Frankia spp., though not traditionally classified as Bacillales, form nitrogen-fixing nodules with actinorhizal non-leguminous plants and produce environmentally robust propagules analogous to endospores.

The ecological versatility of these taxa is reflected in their isolation from soils across diverse biomes: arid deserts, waterlogged paddy fields, boreal forests, saline coastal marshes, and high-altitude alpine meadows. This ecological breadth is not coincidental but reflects the adaptive value of the endospore as an evolutionary innovation that decouples reproductive fitness from momentary environmental favourability.

2.2 Stress Conditions and Tolerance Mechanisms

2.2.1 Thermal Stress

Endospores exhibit extraordinary thermotolerance arising from several structural and biochemical adaptations. The inner membrane of the mature spore is compressed into a gel-like state through dehydration; together with the cortex - a modified peptidoglycan layer and the protein coat, this architecture limits molecular mobility and chemical reactivity. The dipicolinic acid (DPA) - calcium chelate complex, comprising 5–15% of the spore's dry weight, acts as a chemical chaperone, displacing water from macromolecules and stabilizing nucleic acids and proteins against heat-induced denaturation. Alpha/beta-type small acid-soluble proteins (SASPs) bind spore DNA in a sequence-independent manner, conferring resistance to ultraviolet radiation and heat simultaneously. Studies with B. subtilis have shown that SASP-null mutants exhibit markedly reduced thermotolerance, demonstrating the functional primacy of these proteins in spore resistance.

The vegetative cells of PGP Bacillus strains also show notable heat tolerance relative to mesophilic non-spore-forming bacteria. The induction of heat-shock proteins, including the GroEL/GroES chaperonin complex, DnaK/DnaJ/GrpE, and the Clp protease system upon thermal upshift ensures proteome integrity, allowing cells to resume metabolic functions including PGP activities such as phosphatase secretion and IAA biosynthesis at temperatures that would be lethal to many other rhizobacteria.

2.2.2 Drought and Desiccation Stress

Water deficit is the single most significant abiotic constraint on global crop production. Endospore-forming PGPR combat drought stress through multiple strategies operating at different biological scales. At the cellular level, spore dormancy provides absolute protection against desiccation - a critical advantage when soil water potential drops below the threshold for microbial metabolic activity. Upon rewetting, germination is rapidly triggered by specific germinant molecules (L-alanine, inosine, asparagine) acting on GerA, GerB, and GerK receptor complexes in the inner membrane, enabling rapid population recovery. At the exopolymeric level, vegetative cells of B. subtilis, B. amyloliquefaciens, and P. polymyxa secrete exopolysaccharides (EPS) that bind water, form biofilms on root surfaces, and improve soil aggregate stability, collectively reducing hydraulic conductivity loss in the rhizosphere during dry periods. The EPS also promotes root colonization by providing a hydrated microenvironment that facilitates the transfer of bacterially synthesized hormones and nutrients to the plant.

A growing body of literature documents the induction of drought tolerance at the plant level through bacterially produced volatile organic compounds (VOCs), particularly acetoin and 2,3-butanediol, which activate the plant's intrinsic drought-stress signaling pathways including ABA-independent DREB/CBF cascades, leading to stomatal regulation, accumulation of compatible solutes (proline, trehalose, glycine betaine), and upregulation of catalase and superoxide dismutase.

2.2.3 Salinity Stress

Soil salinity, currently affecting over 1 billion hectares globally, imposes osmotic and ionic stress on both plants and their associated microbiota. Halotolerant endospore-forming bacteria have evolved mechanisms to maintain osmotic equilibrium, including the accumulation of compatible solutes (ectoine, hydroxyectoine, trehalose), active sodium extrusion via Na+/H+ antiporters (NhaA, ShaA), and modification of membrane fatty acid composition to maintain fluidity. Notably, certain B. subtilis strains carry the ect operon encoding ectoine biosynthesis enzymes (EctA, EctB, EctC), conferring tolerance to NaCl concentrations exceeding 1.5 M. These osmotic adaptations allow bacterial cells to remain metabolically active and thus capable of PGP functions - at salinity levels that suppress most other rhizobacterial communities.

2.2.4 pH Extremes

Agricultural soils range from pH 3.5 in tropical acidic oxisols to pH 9.0 in calcareous arid soils. Endospore-forming PGPR exhibit broad pH tolerance mediated by proton-pumping ATPases, cytochrome c oxidases with altered proton-coupling stoichiometry, and the alkali-regulated mrp operon encoding a multicomponent $Na^+(K^+)/H^+$ antiporter. Bacillus licheniformis and B. cereus are particularly noted for alkalitolerance, with documented growth across pH 6.5–9.5. Under acidic conditions, the OhrR/OhrB system and thioredoxin-dependent peroxidases mitigate oxidative damage from acid-generated reactive oxygen species. The practical implication is that these bacteria can solubilize mineral phosphates, a pH-dependent process - across the pH range characteristic of most agricultural soils, unlike narrow-range acid phosphatases of strictly acidophilic competitors.

2.2.5 Heavy Metal and Oxidative Stress

Mining runoff, industrial effluents, and improper agrochemical use have introduced elevated heavy metal concentrations into productive soils worldwide. Endospore-forming bacteria exhibit metal resistance through sequestration (metallothioneins, siderophore-mediated chelation), efflux pumping (CzcA/CzcB/CzcC systems for Co_2^+, Zn^{2+}, Cd^{2+}; CopA for Cu^+), and enzymatic detoxification (mercuric reductase encoded by merA). Lysinibacillus sphaericus, in particular, has attracted attention for its capacity to biosorb lead, cadmium, and arsenic on its spore surface, owing to the high density of carboxylate, phosphate, and amine functional groups on the outer coat proteins. This combination of metal resistance and PGP activity positions certain endospore-formers as candidates for phytoremediation-coupled biofertilization strategies.

2.3 Biofertilization Mechanisms

2.3.1 Biological Nitrogen Fixation (BNF)

The conversion of atmospheric dinitrogen (N2) to biologically available ammonium (NH_4^+) is catalyzed by the nitrogenase enzyme complex encoded by the nif gene cluster, under strictly anaerobic or microaerophilic conditions. Among aerobic endospore-formers, P. polymyxa is the most widely studied nitrogen fixer, with nitrogenase activity confirmed by acetylene reduction assays and 15N2 incorporation experiments. The nifH, nifD, and nifK genes encode the iron protein and molybdenum-iron protein subunits, respectively, while regulatory genes nifA and nifL control expression in response to oxygen and fixed nitrogen availability. In Clostridium pasteurianum, the strictly anaerobic nitrogen fixer within Firmicutes, BNF was first characterized and continues to serve as a model for understanding regulatory constraints. Recent metagenomic studies of waterlogged paddy soils suggest that endospore-forming anaerobes contribute meaningfully to the in-situ nitrogen budget, complementing Azotobacter and symbiotic Rhizobium activities.

2.3.2 Phosphate Solubilization

Phosphorus is the second most growth-limiting macronutrient in global agriculture after nitrogen, and its agronomically available form ($H_2PO_4^-$, HPO_4^{2-}) represents only a small fraction of total soil phosphorus, most of which is immobilized as sparingly soluble inorganic salts (hydroxyapatite, tricalcium phosphate, fluorapatite) or organic complexes (phytate, nucleic acids, phospholipids). Bacillus species are among the most efficient mineral phosphate solubilizers, achieving this through the secretion of organic acids - principally gluconic acid (produced by glucose dehydrogenase, GDH), oxalic acid, malic acid, and citric acid - which lower rhizosphere pH and chelate calcium ions, liberating orthophosphate. Studies with B. megaterium WH1 have quantified phosphate solubilization up to 250 mg Pi/L in liquid culture, with significant soil-plant phosphorus transfer documented through 32P isotope dilution assays. Additionally, the secretion of phytases (PhyC in B. amyloliquefaciens, PhyL in B. subtilis) hydrolyzes organic phosphate esters, releasing inositol phosphate fractions from phytate-rich soils common in legume-cultivated fields.

2.3.3 Phytohormone Production

Endospore-forming PGPR synthesize a spectrum of phytohormones that directly modulate plant development. Indole-3-acetic acid (IAA), the predominant natural auxin is produced via the indole-3-pyruvate (IPyA) pathway (encoded by ipdC/ppdC) and the tryptamine pathway, with tryptophan availability in root exudates serving as the primary substrate. IAA at optimal rhizosphere concentrations (10-8 to 10-10 M) promotes primary root elongation, lateral root initiation, and adventitious root formation, collectively expanding the root architecture and improving soil exploration for water and nutrients - effects of particular relevance under drought conditions. Cytokinin production, documented in B. subtilis and P. polymyxa, stimulates shoot growth and delays senescence through trans-zeatin and isopentenyl adenine signaling. Gibberellin biosynthesis, though rarer, has been confirmed in B. pumilus and B. licheniformis, contributing to stem elongation under suboptimal light and temperature conditions. Ethylene precursor (ACC, 1-aminocyclopropane-1-carboxylate) cleavage by ACC deaminase (acdS gene product) in B. subtilis and B. amyloliquefaciens reduces stress ethylene accumulation, which would otherwise trigger senescence and abscission, thereby maintaining plant productivity under saline and drought conditions.

2.3.4 Siderophore Production and Iron Nutrition

Iron, despite its abundance in soil minerals, is largely inaccessible to plants and microbes in aerobic, neutral to alkaline soils due to its precipitation as insoluble $Fe(OH)_3$. Endospore-forming bacteria produce catecholate siderophores (bacillibactin in B. subtilis, schizokinen in B. megaterium) and hydroxamate siderophores with femtomolar iron affinities, sequestering ferric iron (Fe^{3+}) and transporting it via specific outer membrane receptors into the cell. The iron is then transferred to plant roots through siderophore-mediated ligand exchange or direct uptake of Fe-siderophore complexes via plant membrane transporters. Beyond direct iron nutrition, siderophore production limits rhizosphere iron availability to soilborne fungal pathogens (Fusarium, Pythium, Rhizoctonia), constituting a biocontrol mechanism that operates concurrently with biofertilization.

2.3.5 Organic Matter Decomposition and Nutrient Cycling

Bacillus and Paenibacillus species are prolific secretors of hydrolytic enzymes such as cellulases, hemicellulases, proteases, amylases, and lipases that degrade complex organic polymers in crop residues, releasing nitrogen, phosphorus, and sulfur in plant-available forms. This enzymatic catalysis accelerates humus formation, improves soil organic carbon content, and enhances water-holding capacity. In composting systems, thermophilic Bacillus strains (B. thermoamylovorans, B. licheniformis) dominate the active thermophilic phase, driving rapid lignocellulose breakdown. Field applications of Bacillus-based inoculants to incorporate crop residues have consistently documented improved soil enzymatic activity indices (dehydrogenase, urease, arylsulfatase) - surrogate markers of enhanced nutrient cycling, relative to uninoculated controls.

3. Application Studies

3.1 Field and Greenhouse Case Studies

The agronomic value of endospore-forming biofertilizers has been substantiated through an extensive body of controlled experiments and field trials spanning diverse crops, soils, and climatic zones. Table 2 provides a synthesis of representative case studies demonstrating significant plant growth promotion under defined stress conditions.

Table 2. Selected Case Studies of Endospore-Forming Biofertilizers Under Stress Conditions

Crop/Region	Bacterial Strain	Stress Condition	Key Outcomes	Reference
Wheat (Pakistan)	*B. subtilis* SL-13	Drought (30% FC)	+38% shoot biomass, +42% grain yield	Arshad et al., 2017

Crop/Region	Bacterial Strain	Stress Condition	Key Outcomes	Reference
Maize (China)	*B. amyloliquefaciens* FZB42	Salinity (150 mM NaCl)	+29% root elongation, improved K+/Na+ ratio	Chen et al., 2016
Rice (India)	*P. polymyxa* B2	Waterlogging + low N	+25% grain yield, N fixation verified by 15N dilution	Hariprasad & Niranjana, 2009
Chickpea (Iran)	*B. licheniformis* K11	High pH (8.5) + low P	+33% phosphorus uptake, improved nodulation	Zahedi, 2012
Tomato (Brazil)	*B. megaterium* WH1	Heat stress (38°C)	+21% fruit set, enhanced antioxidant enzyme activity	Lopes et al., 2018
Sunflower (Egypt)	*B. subtilis* + *B. megaterium*	Salinity (200 mM NaCl)	+45% oil content, reduced Na+ accumulation	El-Akhdar et al., 2019
Sorghum (Ethiopia)	*P. polymyxa* SC36	Drought + low N soils	+31% biomass, root architecture improvement	Assefa et al., 2020

FC = Field Capacity; N = Nitrogen

3.1.1 Wheat Under Drought Stress (Pakistan)

Arshad et al. (2017) conducted a landmark multi-season field trial in the semi-arid Punjab province of Pakistan, where Bacillus subtilis strain SL-13 — pre-selected for drought tolerance based on in vitro desiccation survival assays — was applied as a seed bacterization treatment to wheat (Triticum aestivum cv. Lasani-08) grown at 30% field capacity. The inoculated plants exhibited a 38% increase in shoot biomass and a 42% increase in grain yield relative to uninoculated drought-stressed controls. Mechanistic attribution studies revealed significant upregulation of genes encoding proline biosynthesis (P5CS), dehydrin accumulation, and improved leaf water potential (ψw) of -1.2 MPa versus -1.8 MPa in controls — effects consistent with IAA-mediated root expansion and EPS-facilitated soil water retention documented in parallel pot studies. Notably, grain protein content was also elevated by 11%, attributable to improved nitrogen assimilation from the inoculated rhizosphere.

3.1.2 Maize Under Salinity Stress (China)

Chen et al. (2016) evaluated the halotolerant strain B. amyloliquefaciens FZB42, the first PGP Bacillus with a completely sequenced genome under hydroponic conditions with 150 mM NaCl, approximating moderately saline irrigated soils. Inoculated maize (Zea mays) seedlings showed a 29% increase in root elongation and a markedly improved K^+/Na^+ ratio in shoot tissue (1.8 versus 0.9 in controls), consistent with bacterially-induced upregulation of high-affinity potassium transporter (HKT1) expression. The study further demonstrated that FZB42 produces iturin A and bacillomycin D under saline conditions, suggesting that salinity does not suppress the biocontrol arm of PGP activity - an important consideration for integrated disease management in salt-affected soils.

3.1.3 Chickpea Under High pH and Phosphorus Limitation (Iran)

Zahedi (2012) investigated the phosphate-solubilizing capacity of B. licheniformis strain K11 in calcareous soils (pH 8.5) representative of much of Iran's agricultural land, where mineral phosphate fertilizer efficiency is critically low. Chickpea (Cicer arietinum) inoculation with K11 resulted in a 33% improvement in shoot phosphorus uptake compared to uninoculated plants receiving equivalent mineral phosphate applications, alongside a significant improvement in nodulation density when co-inoculated with Mesorhizobium ciceri. The authors proposed that K11's gluconic acid secretion locally acidified the rhizosphere by approximately 0.8 pH units, sufficient to convert sparingly soluble tricalcium phosphate into plant-available H2PO4-, while also improving Mesorhizobium survival through altered soil microenvironment conditions.

3.2 Environmental and Economic Implications

The case studies cited above, alongside dozens of analogous investigations from Brazil, India, Egypt, Nigeria, and the Philippines, collectively establish a consistent pattern: endospore-forming PGPR can partially or substantially substitute for synthetic nitrogen and phosphate fertilizer inputs estimated at 20–40% reduction across different cropping systems while maintaining or improving yields under stress conditions that reduce the efficacy of chemical fertilization. The economic ramifications are significant: at a global market value of USD 150 billion for synthetic fertilizers, even a 20% substitution achieved through biologicals would represent a USD 30 billion economic opportunity annually, exclusive of environmental co-benefits such as reduced nitrous oxide emissions, decreased aquifer eutrophication, and improved soil health metrics.

Environmental benefits extend to carbon sequestration. Bacillus-inoculated soils consistently show higher soil organic carbon content, attributable to enhanced root biomass, improved aggregate stability, and accelerated humification of organic residues. Long-term (5–10 year) field trials in Germany and India have documented increases of 0.15–0.35% soil organic carbon in continuously inoculated plots, contributing meaningfully to national soil carbon accounts. The climate-resilience dimension - the ability of spore-based formulations to remain viable under conditions that would eliminate conventional biofertilizer products is particularly relevant in the context of more frequent and severe drought-heat event combinations projected under IPCC climate scenarios.

4. Mechanistic Insights

4.1 Sporulation as an Adaptive Strategy

The molecular cascade governing endospore formation in Bacillus species, the most thoroughly characterized bacterial developmental program, is initiated by the master transcription factor Spo0A upon phosphorylation through a multicomponent phosphorelay involving sensor histidine kinases (KinA–KinE) that respond to nutrient limitation, population density, and DNA damage signals. Phosphorylated Spo0A~P at concentrations above a defined threshold activates transcription of early sporulation genes and simultaneously represses vegetative gene expression, committing the cell to an asymmetric division that produces the forespore and mother cell compartments. Subsequent stage-specific sigma factors (σF, σE in the forespore; σG, σK in the mother cell) orchestrate the sequential assembly of spore structural layers - inner membrane, cortex, coat, exosporium - each conferring distinct protective properties against specific environmental insults.

Critically, the sporulation program incorporates environmental intelligence: the decision to sporulate versus maintain vegetative growth is not simply a starvation response but reflects an integrated assessment of population density (via the SKF/SDP cannibalism peptides and CSF competence-stimulating factor), nutrient quality (via global regulatory networks CcpA for carbon, TnrA/GlnR for nitrogen), and soil moisture (via osmosensing kinases). This regulatory sophistication means that PGP strains do not spontaneously sporulate upon soil application but rather calibrate their developmental state to prevailing conditions — remaining vegetative and metabolically active during periods of optimal soil moisture and nutrient flux, and sporulating in anticipation of deteriorating conditions. The ecological implication is that spore banks in the rhizosphere serve as insurance reservoirs that ensure population persistence, and thus PGP continuity through stress episodes of variable duration.

4.2 Exopolysaccharide-Mediated Soil Interaction

The EPS produced by Bacillus and Paenibacillus species during vegetative growth constitutes a structurally complex, high-molecular-weight matrix whose composition predominantly neutral sugars (glucose, galactose, fructose) interspersed with uronic acids and pyruvate groups endows it with significant water-holding capacity and adhesive properties. EPS-mediated soil aggregation occurs when bacterial biofilms bridge mineral particles, creating stable macroaggregates (>250 μm) with improved porosity and hydraulic conductivity. In drought-prone soils, this aggregation reduces evaporation and maintains a hydrated microenvironment around plant roots even as bulk soil desiccates. Scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) studies using GFP-labelled B. subtilis strains have directly visualized EPS-mediated root biofilm formation, demonstrating preferential colonization of root hair zones and apical meristems — precisely the locations where auxin signalling and nutrient uptake are most active.

4.3 Volatile Organic Compound (VOC) Signalling

Perhaps the most intriguing mechanistic discovery of the past decade in PGPR biology is the characterization of bacterially emitted VOCs as long-distance signalling molecules capable of modulating plant gene expression, metabolism, and stress tolerance without direct physical contact between bacterium and plant. Bacillus subtilis strain GB03 produces a blend of VOCs - dominated by 2,3-butanediol and acetoin - that, when perceived by Arabidopsis thaliana, triggers a systemic signalling cascade: increased cytokinin/auxin ratio driving enhanced shoot branching, HKT1-mediated Na+ exclusion from shoots reducing salt ion toxicity, and HB-1 transcription factor-mediated downregulation of Fe acquisition genes under iron-replete conditions. These gas-phase effects operate at bacterial concentrations orders of magnitude below those required for classical hormone-based PGP, suggesting that VOC-mediated signalling may represent a primary mechanism of PGPR action in field settings where direct root colonization is spatially heterogeneous. The translation of this laboratory phenomenon to field conditions - where VOC dilution, soil gas diffusion limitations, and competitive microbial consumption may attenuate the signal - represents an active research frontier.

4.4 Induced Systemic Resistance (ISR)

Endospore-forming PGPR trigger a salicylate-independent, jasmonate/ethylene-dependent form of broad-spectrum systemic resistance; designated induced systemic resistance (ISR) that primes the plant immune system for more rapid and robust responses to subsequent pathogen or herbivore challenge. Root colonization by *B. amyloliquefaciens* IN937a or B. subtilis GB03 activates NPR1-dependent gene networks in shoots, resulting in enhanced production of pathogenesis-related (PR) proteins, callose deposition at plasmodesmata, and upregulation of WRKY transcription factors governing the defensive transcriptome. Importantly, ISR activation does not impose a significant fitness cost on unstressed plants, unlike constitutively activated SAR but provides measurable protection against a broad spectrum of soilborne pathogens (*Fusarium oxysporum*, *Rhizoctonia solani*, *Pythium ultimum*) and foliar pathogens (*Botrytis cinerea*, *Alternaria alternata*) under both normal and stress conditions.

5. Discussion

5.1 Current State of Knowledge and Validated Findings

The evidence reviewed here firmly establishes endospore-forming bacteria, particularly *Bacillus spp*. and *Paenibacillus spp*. - as scientifically credible, functionally diverse, and practically viable biofertilizer agents. Several findings are now replicated across sufficient independent studies to be considered robust: the phosphate solubilization capacity of B. megaterium and B. subtilis in calcareous soils; the IAA-mediated root architecture enhancement under water deficit; the ACC deaminase-driven stress ethylene reduction in saline environments; and the VOC-induced ISR under diverse pathogen challenges. The thermostability and desiccation resistance of spore formulations, which can maintain viability for 2+ years in commercial carriers at ambient temperature represent a technology advantage that most alternative biofertilizer platforms (Rhizobium, Azospirillum) cannot match.

5.2 Limitations and Knowledge Gaps

Despite these advances, the field is constrained by several critical limitations that temper translational enthusiasm. First and most fundamentally, in vitro PGP activity does not reliably predict field performance. The rhizosphere is a highly competitive, spatially and temporally heterogeneous environment in which introduced strains must compete for nutrients, space, and plant exudate substrates against established, locally adapted indigenous communities. Introduced Bacillus populations frequently decline to ecologically marginal densities within 4–8 weeks of application - a phenomenon termed the 'inoculant survival bottleneck', raising questions about the durability of PGP benefits across a full growing season.

Second, the mechanistic dissection of PGP activity has been conducted predominantly in gnotobiotic or simplified synthetic community systems, where the confounding influences of the native microbiome are eliminated. How endospore-forming PGPR function, and interact within the complex web of fungal, archaeal, viral, and bacterial inhabitants of the bulk and rhizosphere soil remains poorly understood. Recent soil microbiome studies using 16S rRNA amplicon sequencing and shotgun metagenomics have documented that Bacillus inoculation reshapes the native bacterial community composition, in some cases suppressing competing beneficial taxa (e.g., Pseudomonas fluorescens-group strains) while in others facilitating mutualistic associations with mycorrhizal fungi. The net ecological outcome is highly context-dependent and currently not predictable from strain-level genomic data.

Third, the regulatory landscape for biofertilizer registration varies dramatically across jurisdictions, creating barriers to commercialization that disproportionately disadvantage small-scale producers and limit access in precisely the developing-country contexts where sustainable intensification is most urgently needed. Standardized efficacy testing protocols, accepted quality control benchmarks for viable spore counts, and harmonized labelling requirements do not yet exist internationally, complicating comparative evaluation of commercial products.

5.3 Future Research Directions

The most promising near-term avenue for improving the consistency and efficacy of endospore-based biofertilizers lies in the development of synthetic microbial consortia rational combinations of complementary functional strains whose PGP activities are synergistic rather than redundant. Consortia incorporating a phosphate solubilizer (*B. megaterium*), a nitrogen fixer (*P. polymyxa*), an ACC deaminase producer (*B. subtilis*), and a mycorrhiza helper bacterium (*B. pumilus*) could theoretically address the full spectrum of nutritional and stress-related plant requirements simultaneously. Proof-of-concept studies in controlled environments have demonstrated synergistic rather than additive effects in such combinations, though the ecological stability and evolutionary dynamics of multi-strain inoculants require considerably more investigation.

Genomic and metabolomic tools are creating unprecedented opportunities for strain improvement. Comparative genomics of high-performing versus low-performing biofertilizer strains has identified candidate PGP gene clusters whose expression is correlated with field efficacy, enabling targeted selection from natural diversity. CRISPR-Cas9-mediated genome editing now feasible in *B. subtilis* which offers the prospect of rationally enhancing specific PGP traits (e.g., upregulating phytase secretion, improving ACC deaminase thermostability) without the regulatory concerns associated with classical GMO approaches, if contained within defined application frameworks. Metabolomics-guided profiling of rhizosphere exudate responses to inoculant application can serve as an early-harvest indicator of PGP activity, reducing the time and cost of strain selection from years to months.

Formulation technology represents another frontier with substantial practical impact. Nanoencapsulation of Bacillus spores within biodegradable polymer matrices (alginate, chitosan, polyhydroxyalkanoates) protects against UV radiation and chemical pesticides during field application while enabling controlled-release germination triggered by soil moisture and temperature cues. Carrier medium optimization moving from conventional peat-based formulations to mineral-based superabsorbent polymers or biochar, can extend shelf life, improve spore-to-soil transfer efficiency, and provide additional soil amendment benefits. Integration with precision agriculture platforms using remote sensing to identify spatially variable stress zones within fields and deploying variable-rate biofertilizer application accordingly. It could significantly improve cost-effectiveness by concentrating biological inputs where they are most needed.

6. Conclusion

Endospore-forming bacteria represent a uniquely valuable biological resource at the intersection of evolutionary adaptation and agricultural necessity. Their defining capacity to form environmentally resistant spores addresses one of the primary obstacles to biofertilizer adoption product instability and inconsistent field performance, while their multifaceted plant growth-promoting activities encompass virtually the full spectrum of nutritional and stress-alleviation functions that crops require. The evidence synthesized in this review demonstrates that:

1. Species including *B. subtilis*, *B. amyloliquefaciens*, *B. megaterium*, and *P. polymyxa* consistently demonstrate PGP activity across diverse stress conditions including drought, salinity, thermal extremes, and pH stress.
2. Biofertilization mechanisms such as nitrogen fixation, phosphate solubilization, phytohormone production, siderophore secretion, and VOC emission are molecularly well-characterized and operate synergistically to improve plant fitness.
3. Field-scale evidence supports yield benefits of 21–45% in stressed crops across multiple continents and cropping systems, with concurrent reductions in required chemical fertilizer inputs.
4. Molecular mechanisms including EPS-mediated soil aggregation, SASP-mediated spore DNA protection, and ACC deaminase-driven ethylene regulation are mechanistically validated and provide a sound biological rationale for the observed agronomic effects.
5. Critical limitations, particularly inoculant survival bottlenecks, variable field performance, and regulatory inconsistencies - must be systematically addressed before the full potential of these organisms can be realized in commercial agriculture.

The imperative to transition agriculture toward reduced chemical dependency and enhanced climate resilience makes the development of endospore-based biofertilizers not merely a scientific opportunity but an urgent societal priority. Future research investments should prioritize multi-strain consortium development, precision formulation technologies, long-term field monitoring under realistic climate scenarios, and the creation of harmonized international regulatory frameworks that facilitate, rather than impede the global deployment of these remarkable microorganisms in service of food security.

Conflict of interest

The authors declare no conflict of interest

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CHAPTER 12: Pharmacophore Modeling: Concepts and Applications in Drug Development

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Abstract

Pharmacophore modeling is a cornerstone of computer-aided drug design (CADD), enabling researchers to define the essential molecular features responsible for biological activity and to rapidly screen large chemical libraries for potential drug candidates. This review provides a comprehensive, evidence-based overview of the theoretical foundations, methodological developments, and practical applications of pharmacophore modeling in drug development. We discuss the evolution from static three-dimensional (3D) pharmacophore models to dynamic pharmacophore (dynophore) approaches derived from molecular dynamics (MD) simulations, and critically appraise the integration of artificial intelligence and machine learning (AI/ML) into pharmacophore-based workflows. Structure-based and ligand-based approaches are systematically examined alongside leading software platforms. Applications across diverse therapeutic areas—including COVID-19, oncology, tuberculosis, neurodegenerative diseases, and cardiovascular medicine—are presented with recent case studies. Current challenges encompassing molecular flexibility, solvation effects, data quality, and selectivity are critically examined. Emerging trends in AI-driven pharmacophore generation and multi-target drug design are discussed, highlighting the future trajectory of the field.

Keywords: pharmacophore modelling, computer-aided drug design (CADD), virtual screening, ligand-based drug design, structure-based drug design, molecular dynamics, machine learning, QSAR, drug discovery

1. Introduction

Drug discovery is an extraordinarily complex, costly, and time-consuming endeavor, with attrition rates of drug candidates remaining alarmingly high. Computational approaches, collectively termed computer-aided drug design (CADD), have emerged as indispensable tools to accelerate early-phase drug discovery, reduce costs, and improve success rates (Giordano et al., 2022). Among these strategies, pharmacophore modeling holds an enduring and central position.

A pharmacophore is formally defined by the International Union of Pure and Applied Chemistry (IUPAC) as ‘the ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target structure and to trigger or to block its biological response’ (Madzhidov et al., 2020). In practical terms, a pharmacophore model encodes the spatial arrangement of chemical features—such as hydrogen bond donors, hydrogen bond acceptors, hydrophobic regions, aromatic rings, and ionizable groups—shared among bioactive molecules and critical for binding to a target.

The utility of pharmacophore models is multifaceted: they can be used prospectively to screen virtual chemical libraries and identify novel hit compounds, or retrospectively to rationalize structure–activity relationships (SAR) and guide lead optimization (Elsaka et al., 2026). The field has grown substantially: recent algorithmic advances in machine learning, MD simulations, and freely accessible web servers continue to make 3D pharmacophore modeling a vibrant tool in drug design (Schaller et al., 2020).

This review provides academic researchers with a rigorous synthesis of pharmacophore modeling covering its conceptual foundations, algorithmic underpinnings, software ecosystem, integration with complementary computational methods, and applications across therapeutic areas.

2. Historical Background and Evolution of the Pharmacophore Concept

The intellectual lineage of pharmacophore modeling begins with Paul Ehrlich’s receptor theory at the turn of the twentieth century. Ehrlich’s recognition that molecules possess discrete regions responsible for biological activity laid the epistemological groundwork for modern drug design. Subsequent quantification of drug–receptor interactions by Alfred Clark introduced thermodynamic principles that foreshadowed quantitative structure–activity relationship (QSAR) methods.

The computational formalization of the pharmacophore concept gained momentum in the 1970s with the work of Peter Gund, who proposed the first geometric definition and developed algorithmic approaches to identify common three-dimensional arrangements of functional groups. The 1980s and 1990s saw the development of landmark programs—DISCO, CATALYST, and GASP—that established algorithmic foundations still reflected in contemporary tools. The introduction of excluded volumes to represent steric clashes with the receptor substantially increased the discriminative power of pharmacophore models (Giordano et al., 2022).

The 2000s witnessed maturation of pharmacophore modeling into a mainstream CADD tool, driven by improvements in computational hardware, the explosion of protein structure data, and the availability of large commercial and public chemical databases. Recent advances—particularly the integration of molecular dynamics (MD) simulations to generate dynamic pharmacophores (‘dynophores’), and the incorporation of AI/ML—represent the current frontier of the field (Schaller et al., 2020; Elsaka et al., 2026).

3. Theoretical Foundations

3.1 Definition and Components of a Pharmacophore

A pharmacophore model comprises a set of chemical features and their spatial relationships, defined within a three-dimensional coordinate framework. The core chemical features recognized by most platforms include (Giordano et al., 2022; Elsaka et al., 2026):

- Hydrogen Bond Donors (HBD): atoms capable of donating a hydrogen bond, typically N–H or O–H groups.
- Hydrogen Bond Acceptors (HBA): electronegative atoms capable of accepting a hydrogen bond (e.g., carbonyl oxygens, aromatic nitrogens).
- Hydrophobic Centers (HYD): non-polar regions engaging van der Waals interactions with lipophilic binding site pockets.
- Aromatic Rings (AR): planar aromatic systems capable of pi–pi stacking and cation–pi interactions.
- Positive Ionizable (PI) and Negative Ionizable (NI) Groups: regions carrying or capable of acquiring a formal charge, participating in salt-bridge interactions.
- Excluded Volumes (EV): spherical regions representing receptor atoms that ligands must avoid to bind productively.

Each feature is defined by a centroid coordinate, a tolerance radius representing permissible positional variation across ligands, and in some implementations a vector defining directionality (e.g., hydrogen bond direction). The pharmacophore model as a whole defines the minimal subset of features and their geometric relationships required for biological activity (Madzhidov et al., 2020).

3.2 Conformational Analysis and the Bioactive Conformation

A fundamental challenge in 3D pharmacophore modeling is the conformational flexibility of drug-like molecules. The bioactive conformation—the 3D arrangement adopted by the ligand upon target binding—may differ substantially from the lowest-energy conformer in solution. Accurate pharmacophore generation therefore requires thorough conformational sampling of all training compounds (Polishchuk et al., 2019). Common algorithms include systematic torsional searches, Monte Carlo methods, and MD-based sampling, each offering a trade-off between conformational space coverage and computational cost.

3.3 Molecular Alignment and Superimposition

Pharmacophore model generation from multiple ligands requires identification of a common spatial arrangement of pharmacophoric features—a constrained molecular alignment problem. Alignment methods can be feature-based (aligning chemical feature centroids) or field-based (aligning molecular property fields such as electrostatic potential and shape). Genetic algorithms, simulated annealing, and exhaustive search methods have all been applied to the pharmacophore alignment problem (Schaller et al., 2020).

4. Pharmacophore Modeling Approaches

4.1 Ligand-Based Pharmacophore Modeling (LBPM)

Ligand-based pharmacophore modeling (LBPM) is employed when structural information about the target protein is unavailable or unreliable. The fundamental assumption is that structurally diverse molecules sharing a common biological activity do so through a shared set of pharmacophoric interactions with the same binding site (Giordano et al., 2022). The LBPM workflow begins with curation of a training set comprising active and, where available, inactive compounds. Conformational ensembles are generated, followed by pharmacophore hypothesis generation via alignment and feature extraction algorithms.

Quality assessment of LBPM models is typically performed through cross-validation. Metrics such as the Enrichment Factor (EF), Receiver Operating Characteristic (ROC) curve area, and Boltzmann-Enhanced Discrimination of ROC (BEDROC) are used to quantify the model's discriminative ability (Madzhidov et al., 2020). Madzhidov et al. (2020) proposed a probabilistic approach to pharmacophore-based virtual screening using multiple pharmacophore models, demonstrating superior early enrichment compared to conventional single-model approaches.

4.2 Structure-Based Pharmacophore Modeling (SBPM)

Structure-based pharmacophore modeling (SBPM) leverages the three-dimensional structure of the target protein, typically obtained from X-ray crystallography, NMR spectroscopy, or cryo-electron microscopy. The pharmacophore is derived by analyzing protein–ligand interactions in the binding site (Rampogu& Lee, 2021). In protein–ligand complex-based SBPM, hydrogen bonds, hydrophobic contacts, and ionic interactions between the ligand and protein residues are translated directly into pharmacophoric features. SBPM is sensitive to the quality and resolution of the protein structure, the choice of protein conformation, and the treatment of solvation and induced-fit effects.

4.3 Dynamic and Hybrid Pharmacophore Approaches

Dynamic pharmacophore models (dynophores) account for receptor flexibility by deriving pharmacophore features from molecular dynamics (MD) simulations of the protein–ligand complex. Dynophores sequentially extract interaction points from each MD trajectory frame, producing super-features statistically characterized by occurrence frequency and volumetric density clouds (Schaller et al., 2020). Ensemble-based approaches—generating models from multiple protein snapshots—capture conformational heterogeneity of the binding site and are more tolerant of receptor flexibility.

Polishchuk et al. (2019) demonstrated that pharmacophores retrieved from protein–ligand MD trajectories outperform those derived from single crystal complex structures for virtual screening. Their conformer coverage approach for ranking compounds across multiple pharmacophore models showed superior performance for CDK2 ligand identification, illustrating the practical advantage of dynamic approaches (Polishchuk et al., 2019).

Choudhury and Bhardwaj (2020) introduced a hybrid dynamic pharmacophore model (DHPM) methodology combining static SBPM with dynamic MD-derived components for anti-tuberculosis drug design. This approach yielded novel chemotypes targeting *Mycobacterium tuberculosis* dihydrodipicolinate reductase (Mtb-DapB), demonstrating the value of hybrid strategies for targets with significant conformational flexibility (Choudhury & Bhardwaj, 2020).

5. Software Tools and Platforms

A wide array of commercial and open-source software tools supports pharmacophore model generation, visualization, and virtual screening. A summary of the most widely used platforms is presented in Table 1. The selection and application of software tools should be guided by the availability of target structural data, the size of the compound library to be screened, and the computational resources available (Giordano et al., 2022).

Table 1. Major Pharmacophore Modeling Software Platforms

Software	Type	Approach	Key Features
LigandScout	Commercial	LB / SB	Automated feature perception; multi-conformer screening; interaction diagram export
Schrödinger Phase	Commercial	LB / SB	Integrated VS workflow; HipHop/HypoGen algorithms; FEP+ coupling
MOE (CCG)	Commercial	LB / SB	Flexible scripting; SBPM from binding site; pharmacophore-constrained docking
CATALYST/DS	Commercial	LB / SB	HipHop and HypoGen; legacy but widely cited in literature
ROCS (OpenEye)	Commercial	Shape+Feature	Shape and color (feature) overlay for 3D alignment and screening
PharmaGist	Free / Web	LB	Web-based flexible alignment; no installation required
Pharmer	Open-source	LB / SB	kd-tree indexing; ultra-fast large-database screening
PyRod	Open-source	SB (MD)	Water MD hotspot-based pharmacophore generation
Open Babel	Open-source	Utility	Format interconversion; conformer generation pipeline

The quantitative pharmacophore activity relationship (QPhAR) method, implemented within LigandScout, extends classical pharmacophore modeling by introducing quantitative activity weights to pharmacophoric features, enabling activity prediction in addition to binary active/inactive classification (Kohlbacher et al., 2022). Kohlbacher et al. (2022) demonstrated the utility of QPhAR for virtual screening and lead optimization across multiple target classes, confirming its advantage over conventional pharmacophore approaches in enrichment and predictive accuracy.

In the GPCR domain, Noonan et al. (2022) reviewed the integration of 3D pharmacophore modeling with AI approaches for deciphering GPCR pharmacology, covering applications in de novo drug design, biased ligand discovery, allosteric modulation, and scaffold hopping. Their analysis highlights how GPCR structural biology and cheminformatics can be synergistically combined through pharmacophore tools (Noonan et al., 2022).

6. Integration with Complementary Computational Methods

6.1 Virtual Screening Cascades

In contemporary drug discovery, pharmacophore modeling functions as a component of multi-stage virtual screening (VS) cascades. A typical cascade applies physicochemical property filters (e.g., Lipinski’s Rule of Five) to large chemical databases, followed by pharmacophore screening as an efficient second-stage filter, then molecular docking, and finally free energy calculations for top-ranked candidates (Giordano et al., 2022). Pharmacophore screening, with its sub-second per-compound screening times, provides a mechanistically informed filter far more selective than simple property-based filters but far less computationally demanding than docking.

6.2 Integration with Molecular Docking

Molecular docking and pharmacophore modeling are highly complementary. Pharmacophore constraints can be incorporated directly into docking algorithms as geometric constraints, biasing the search toward poses satisfying pharmacophoric requirements. This pharmacophore-constrained docking reduces conformational search space, improves pose prediction accuracy, and enhances enrichment of true actives (Tian et al., 2022; Rampogu& Lee, 2021).

6.3 QSAR and 3D-QSAR

Pharmacophore models share conceptual and methodological overlap with three-dimensional QSAR methods such as CoMFA and CoMSIA. Pharmacophore-based alignments have frequently served as the structural basis for 3D-QSAR studies, where the pharmacophore model defines the alignment hypothesis while the QSAR model quantifies the relationship between molecular fields and biological activity. Banerjee et al. (2024) applied this combined approach to SARS-CoV-2 3CL^{OOO} inhibitors, identifying the importance of hydrogen bond acceptor groups at the S2 and S1’ pockets through integrated pharmacophore mapping and field-based 3D-QSAR (Banerjee et al., 2024).

6.4 Machine Learning and Artificial Intelligence

The integration of AI/ML with pharmacophore modeling represents one of the most dynamic frontiers in the field. Elsaka et al. (2026) reviewed how recent advances include multi-pharmacophore strategies that better capture ligand diversity and target flexibility, as well as dynamic pharmacophore models derived from MD simulations that reflect time-dependent interaction patterns. The integration of AI/ML has further improved feature extraction, virtual screening accuracy, and predictive performance across discovery pipelines (Elsaka et al., 2026).

Hayek-Orduz et al. (2025) developed dyphAI, an innovative tool integrating ML models, ligand-based pharmacophore models, and complex-based pharmacophore models into an ensemble for identifying novel acetylcholinesterase (AChE) inhibitors relevant to Alzheimer’s disease. From ZINC database screening, nine candidate molecules were experimentally tested, with two showing IC$_{50}$ values equal to or lower than galantamine—a clinically used AChE inhibitor—demonstrating a rigorous bench-to-bench validation cycle (Hayek-Orduz et al., 2025).

7. Applications in Drug Development

7.1 COVID-19 and Infectious Diseases

The urgency of the COVID-19 pandemic catalyzed widespread application of pharmacophore-based drug repurposing approaches targeting SARS-CoV-2 proteins. Rampogu and Lee (2021) employed SBPM from the SARS-CoV-2 nsp16/nsp10 complex to screen the DrugBank database, identifying framycetin, kanamycin, and tobramycin as promising candidates with higher docking scores than the reference compound S-adenosyl methionine and repurposing drugs remdesivir and hydroxychloroquine (Rampogu& Lee, 2021).

Tian et al. (2022) used SBPM combined with molecular docking to screen an in-house database of 35,000 compounds against SARS-CoV-2 papain-like protease (PLpro). Biological evaluation confirmed several hit compounds as potent PLpro inhibitors, with MD simulations validating the stability of the predicted complexes (Tian et al., 2022).

Banerjee et al. (2024) applied structure-based pharmacophore mapping and molecular docking-dependent QSAR studies to SARS-CoV-2 3CL^{OOO} inhibitors, identifying Elobixibat as a promising drug candidate for repurposing, with MD simulation results supporting its binding stability at the 3CL^{OOO} active site (Banerjee et al., 2024).

In tuberculosis drug discovery, Choudhury and Bhardwaj (2020) demonstrated that hybrid dynamic pharmacophore models derived from MD simulations for Mtb-DapB successfully identified novel chemotypes from unexplored chemical space, representing an advance over conventional static SBPM for drug-resistant mycobacterial targets (Choudhury & Bhardwaj, 2020). Alotaibi (2024) combined pharmacophore generation with MD simulation and virtual screening of phytochemical databases to identify promising inhibitors of the mutant ribosomal protein subunit RpsA in multidrug-resistant tuberculosis, yielding 176 initial hits subsequently refined by docking and MD validation (Alotaibi, 2024).

7.2 Neurodegenerative Diseases

Alzheimer’s disease (AD) represents a major application domain for pharmacophore-based drug design. Hayek-Orduz et al. (2025) developed the dyphAI ensemble integrating dynamic pharmacophore modeling with AI for AChE inhibitor discovery. Their protocol captured key protein–ligand interactions including π–cation interactions with Trp-86 and π–π interactions with Tyr-341, Tyr-337, Tyr-124, and Tyr-72, identifying 18 novel hits from the ZINC database with binding energies ranging from −62 to −115 kJ/mol (Hayek-Orduz et al., 2025). Experimental validation confirmed two molecules—P-1894047 and P-2652815—as potent AChE inhibitors, illustrating the power of AI-augmented dynamic pharmacophore methods for CNS drug discovery.

7.3 Oncology

Pharmacophore modeling has found extensive application in oncology, particularly for kinase inhibitor design and epigenetic target modulation. The conserved ATP-binding pocket of kinases provides a tractable pharmacophore framework, with type I, II, and III inhibitor pharmacophores encoding distinct interaction patterns. Quantitative pharmacophore activity relationship (QPhAR) methods have been applied to optimize kinase inhibitor selectivity profiles (Kohlbacher et al., 2022).

7.4 GPCR Drug Discovery

G protein-coupled receptors (GPCRs) represent the largest class of drug targets, responsible for approximately 34% of all FDA-approved drugs. Noonan et al. (2022) comprehensively reviewed the application of 3D pharmacophore modeling to GPCR drug discovery, highlighting successes in scaffold hopping, biased ligand identification, GPCR de-orphanization, and mechanistic elucidation of ligand–receptor interactions. Advances in incorporating molecular dynamics and machine learning were highlighted as key drivers of future GPCR pharmacophore research (Noonan et al., 2022).

8. Current Challenges and Limitations

8.1 Molecular Flexibility and Conformational Bias

Molecular flexibility remains the most significant methodological challenge in pharmacophore modeling. Both ligands and receptors exhibit conformational variability that complicates the generation of fixed-geometry models. On the ligand side, identification of the bioactive conformation from the conformational ensemble is non-trivial, particularly for highly flexible molecules. On the receptor side, induced-fit effects can substantially alter the binding site geometry between unliganded and ligand-bound states (Elsaka et al., 2026). Conformational bias—in which the pharmacophore model reflects the conformation of the template ligand rather than the true bioactive geometry—remains a persistent limitation of LBPM.

Ensemble-based pharmacophore approaches, dynamic pharmacophore models, and flexible pharmacophore query tools represent partial solutions. Polishchuk et al. (2019) showed that MD-derived pharmacophore ensembles outperform single-crystal-derived models for CDK2 virtual screening, underscoring the practical value of dynamic approaches for flexible targets (Polishchuk et al., 2019).

8.2 Water and Solvation Effects

Structural water molecules at the binding site can serve as hydrogen bond bridges between ligand and receptor, effectively extending the pharmacophoric network. Displacing or conserving such water molecules upon ligand binding carries significant thermodynamic consequences not captured by conventional pharmacophore feature sets. Emerging approaches incorporate explicit water pharmacophore features or use WaterMap-type analyses to identify thermodynamically favorable water positions (Schaller et al., 2020).

8.3 Data Quality and Training Set Composition

The predictive quality of pharmacophore models critically depends on the quality and representativeness of the training data. Assay variability between laboratories, activity cliffs, stereochemical ambiguity, and the paucity of truly inactive compounds all degrade model quality. Careful curation—including chemical structure standardization, activity unit normalization, and removal of assay artifacts—is a prerequisite for reliable pharmacophore model generation (Madzhidov et al., 2020; Elsaka et al., 2026).

8.4 Selectivity and Multi-Target Pharmacophore Design

Designing pharmacophore models that capture selectivity—distinguishing the desired target from closely related off-targets—is particularly challenging for protein families with conserved binding site architectures such as kinases, GPCRs, and nuclear receptors. Selectivity pharmacophores using information from both desired targets and anti-targets represent an important frontier. Kohlbacher et al. (2022) addressed this through the QPhAR framework, which enables quantitative selectivity optimization within the pharmacophore model (Kohlbacher et al., 2022).

9. Future Directions and Emerging Trends

9.1 Artificial Intelligence-Driven Pharmacophore Generation

The convergence of pharmacophore modeling with deep learning is transforming the field. Graph neural network architectures trained on large pharmacological datasets can learn implicit pharmacophoric representations that generalize across chemical series. Elsaka et al. (2026) reviewed how AI/ML integration has improved feature extraction, virtual screening accuracy, and predictive performance, while noting that interpretability—a defining strength of classical pharmacophore models—must be preserved in AI-augmented approaches (Elsaka et al., 2026).

Generative AI approaches, including diffusion models and variational autoencoders, are being applied to pharmacophore-conditioned molecular generation—producing novel structures satisfying predefined pharmacophoric constraints. This pharmacophore-guided generative design paradigm offers a principled approach to scaffold hopping and exploration of novel chemical space beyond the training data of conventional virtual screening campaigns (Noonan et al., 2022).

9.2 Multi-Target Drug Design

The polypharmacology paradigm recognizes that many effective drugs exert therapeutic effects through interactions with multiple targets. Multi-target pharmacophore design—constructing models that simultaneously satisfy pharmacophoric requirements of multiple targets—is gaining prominence in oncology, where combination effects and resistance mechanisms demand multi-target strategies. Computational frameworks for multi-target pharmacophore optimization represent an active and growing research area (Elsaka et al., 2026).

9.3 Integration with Structural Genomics and Chemical Biology

The integration of pharmacophore modeling with chemical proteomics and activity-based protein profiling (ABPP) is enabling the design of pharmacophore-based probes for chemoproteomics applications, including the identification of novel drug targets and profiling of compound selectivity across the proteome. This systems-level perspective represents a significant expansion of the traditional single-target pharmacophore paradigm (Schaller et al., 2020).

10. Conclusion

Pharmacophore modeling has demonstrated remarkable longevity and adaptability as a computational tool in drug discovery. From its conceptual origins in Paul Ehrlich’s receptor theory to its current incarnation as an AI-augmented component of integrated drug discovery platforms, the field has continuously evolved in response to new methodological developments. The unique combination of structural interpretability, computational efficiency, and predictive utility positions pharmacophore modeling as an indispensable element of the modern drug discoverer’s arsenal (Giordano et al., 2022; Schaller et al., 2020).

Recent advances—including dynamic pharmacophore (dynophore) models, multi-pharmacophore strategies, quantitative pharmacophore activity relationships (QPhAR), and AI/ML integration—have substantially expanded the predictive power and applicability of pharmacophore-based workflows (Elsaka et al., 2026; Kohlbacher et al., 2022; Schaller et al., 2020). Addressing persistent challenges in molecular flexibility, solvation, and multi-target selectivity will require sustained methodological innovation.

As demonstrated by applications in COVID-19, tuberculosis, Alzheimer’s disease, GPCR-targeting drug discovery, and oncology, pharmacophore modeling—enriched by the data and algorithmic resources of the twenty-first century—will remain a vital engine of rational drug design for the foreseeable future (Rampogu& Lee, 2021; Choudhury & Bhardwaj, 2020; Hayek-Orduz et al., 2025; Noonan et al., 2022).

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CHAPTER 13: Biopackaging Technology: Advances, Materials and Future Directions

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Abstract: Biopackaging technology has emerged as a sustainable alternative to conventional petroleum-based packaging, driven by increasing environmental concerns, regulatory pressures, and consumer demand for eco-friendly materials. This chapter provides a comprehensive overview of biopackaging systems, focusing on biodegradable, bio-based, and edible packaging materials derived from renewable resources such as polysaccharides, proteins, and lipids. The chapter explores recent advances in material engineering, including nanocomposites, active packaging, and smart packaging systems that enhance shelf life, safety, and quality of food products. Emphasis is placed on biopolymers such as polylactic acid (PLA), polyhydroxyalkanoates (PHA), starch-based films, and cellulose derivatives, along with their physicochemical and barrier properties. Additionally, innovations in antimicrobial and antioxidant packaging using natural bioactive compounds are discussed. Despite significant progress, challenges such as cost, scalability, mechanical limitations, and regulatory constraints remain critical barriers to widespread commercialization. The chapter critically evaluates these limitations and highlights future directions, including integration with nanotechnology, circular economy models, and intelligent packaging systems. Overall, biopackaging represents a transformative approach toward sustainable materials science and food technology, offering promising solutions to global plastic pollution and environmental-sustainability.

Keywords: Biopackaging, Biopolymers, Sustainable materials, Active packaging, Nanocomposites, Food packaging.

1. Introduction

The exponential growth in global plastic production and its environmental consequences have intensified the search for sustainable alternatives in packaging industries. Conventional petroleum-based plastics are persistent, non-biodegradable, and contribute significantly to environmental pollution, particularly in marine ecosystems. Biopackaging technology has emerged as a promising solution, leveraging renewable biological resources to develop environmentally friendly packaging materials (Gómez-Gast et al., 2022).

Biopackaging refers to packaging systems derived from natural or bio-based materials that are biodegradable, compostable, or edible (Versino et al., 2023). These materials include polysaccharides (e.g., starch, cellulose, chitosan), proteins (e.g., gelatin, casein), and lipids (e.g., waxes), as well as synthetic biopolymers such as polylactic acid (PLA) and polyhydroxyalkanoates (PHA). The shift toward such materials is supported by global sustainability initiatives and policies aimed at reducing plastic waste (Shaikh 2021; Teleky et al., 2025).

Recent advancements in material science have enabled the development of functional biopackaging systems, including active and intelligent packaging (Debeaufort, 2021). These systems not only serve as barriers but also interact with food products to enhance shelf life and safety. Furthermore, nanotechnology has significantly improved the mechanical and barrier properties of biopolymers (Reichert et al., 2020).

2. Biopackaging Materials

Biopackaging materials are derived from renewable biological sources and are designed to be biodegradable, compostable, or edible. These materials have gained significant attention as sustainable alternatives to conventional petroleum-based plastics due to their reduced environmental impact and compatibility with circular economy principles. Based on their origin and chemical composition, biopackaging materials can be broadly classified into natural biopolymers and bio-based synthetic polymers. Each class exhibits distinct physicochemical, mechanical, and barrier properties that influence their applicability in packaging systems (Versino et al., 2023).

2.1 Polysaccharide-Based Materials

Polysaccharides are among the most widely used biopolymers in packaging due to their abundance, low cost, and excellent film-forming ability. These materials are primarily hydrophilic, which contributes to good oxygen barrier properties but limits their resistance to moisture (Kurczewska et al., 2022; Salvay, 2025).

- Starch-Based Materials: Starch is a naturally occurring polymer composed of amylose and amylopectin. It is extensively used in biodegradable packaging films due to its availability and low cost. Thermoplastic starch (TPS) can be produced through plasticization, enabling its use in flexible films. However, starch-based materials exhibit poor mechanical strength and high sensitivity to moisture, which necessitates blending with other polymers or reinforcement with fillers (Amaraweera et al., 2021).
- Cellulose and Its Derivatives: Cellulose is the most abundant biopolymer on Earth and offers excellent mechanical strength and chemical stability. Derivatives such as cellulose acetate, carboxymethyl cellulose (CMC), and nanocellulose (cellulose nanocrystals and nanofibrils) are widely used in packaging applications. Nanocellulose, in particular, has gained attention due to its high surface area, reinforcing capability, and ability to improve barrier properties.
- Chitosan: Chitosan is derived from chitin, typically obtained from crustacean shells. It is biodegradable, biocompatible, and possesses inherent antimicrobial properties, making it highly suitable for active packaging applications. Chitosan-based films are effective in inhibiting microbial growth, although their mechanical strength and water resistance require enhancement through composite formation (Abdelhamid et al., 2022).

2.2 Protein-Based Materials

Proteins are another important class of biopolymers used in packaging due to their ability to form cohesive, flexible films with good gas barrier properties.

- Animal-Derived Proteins: Gelatin, casein, and whey proteins are commonly used in film formation. These materials provide excellent oxygen barrier properties and transparency. However, they are highly sensitive to humidity and may undergo structural changes under varying environmental conditions.
- Plant-Derived Proteins: Soy protein, zein (from corn), and gluten are widely explored for packaging applications. These proteins offer good film-forming capabilities and are renewable and biodegradable. However, similar to animal proteins, they require modifications to improve water resistance and mechanical stability (Tanuhe et al., 2025).

2.3 Lipid-Based Materials

Lipid-based materials are primarily used to enhance the moisture barrier properties of biopackaging systems. These include waxes, fatty acids, and glycerides. Lipids are often used as coatings or incorporated into composite films. They significantly reduce water vapor permeability. However, lipid-based films generally exhibit poor mechanical strength and brittleness. To overcome these limitations, lipids are typically combined with polysaccharides or proteins to form multilayer or composite structures (Antorana et al., 2025).

2.4 Bio-Based Synthetic Polymers

In addition to natural biopolymers, several synthetic polymers derived from renewable resources have been developed for biopackaging applications (Cottet et al., 2020).

- Polylactic Acid (PLA): PLA is one of the most widely used bio-based polymers, produced from fermented plant sugars such as corn starch. It is compostable under industrial conditions and exhibits good transparency and mechanical properties. However, PLA is relatively brittle and has limited thermal resistance.
- Polyhydroxyalkanoates (PHA): PHAs are a family of biodegradable polyesters synthesized by microorganisms. They are fully biodegradable in various environments, including soil and marine conditions. PHAs exhibit properties comparable to conventional plastics but are currently limited by high production costs.
- Polybutylene Succinate (PBS) and Other Biopolyesters: PBS and related polymers are emerging as promising alternatives due to their biodegradability and improved flexibility compared to PLA. These materials are often used in blends to enhance overall performance (Tan et al., 2021).

3. Composite and Nanocomposite Materials

To address the inherent limitations of individual biopolymers, composite materials have been developed by combining different polymers or incorporating reinforcing agents.

3.1 Polymer Blends: Blending different biopolymers can improve mechanical strength, flexibility, and barrier properties. For example, starch–PLA blends combine biodegradability with improved structural integrity.

3.2 Nanocomposites: Nanomaterials such as nanocellulose, nanoclays, and metal nanoparticles are incorporated into biopolymer matrices to enhance performance. Nanocellulose improves tensile strength and reduces gas permeability. Nanoclays enhance thermal stability and barrier properties. Metal nanoparticles (e.g., silver, zinc oxide) provide antimicrobial functionality. Nanocomposites represent a significant advancement in biopackaging, enabling materials to meet industrial performance standards (Grzejda, 2025, Musa et al., 2025). Nanotechnology has enhanced biopackaging properties significantly (Satchanska et al., 2024), where nano-enabled systems dynamically support antimicrobial activity.

4. Edible Films and Coatings

Edible packaging materials are designed to be consumed along with the food product, eliminating packaging waste entirely. Typically composed of polysaccharides (alginate, starch), proteins (gelatin), or lipids, they represent an entirely zero-waste structural configuration.

- Often enriched with functional additives such as vitamins, antioxidants, or antimicrobial agents.
- Commonly used for protecting fruits, vegetables, and confectionery products.

These materials offer a unique approach to sustainability, although challenges remain in terms of durability and consumer acceptance (Gheorghita et al., 2020).

5. Current Status of Biopackaging and Next-Generation Materials

Biopackaging technology has transitioned from a niche research domain to a rapidly expanding industrial sector, driven by increasing environmental concerns, regulatory mandates, and consumer awareness. Conventional petroleum-based plastics, which account for a significant proportion of global waste, have prompted urgent efforts toward the development of sustainable alternatives. In this context, biopackaging, defined as packaging derived from renewable biological resources and/or possessing biodegradability, has gained considerable traction.

Currently, the global biopackaging market is experiencing steady growth, supported by governmental policies such as bans on single-use plastics and incentives for biodegradable materials. Commercially available biopackaging solutions include materials like polylactic acid (PLA), starch-based plastics, and cellulose-derived films, which are already being used in food packaging, disposable containers, and agricultural applications. Major food and beverage companies are increasingly adopting such materials to align with sustainability goals.

Despite this progress, the widespread adoption of biopackaging is still constrained by several factors. These include higher production costs compared to conventional plastics, limited mechanical strength, poor moisture barrier properties, and challenges in large-scale manufacturing. Additionally, inconsistencies in biodegradability standards and lack of industrial composting infrastructure limit the practical environmental benefits of these materials (Venkatesan et al., 2025).

Recent advancements have focused on improving the functional properties of biopackaging through material blending, chemical modification, and incorporation of nanomaterials. The integration of active and intelligent packaging technologies has further expanded the scope of biopackaging from passive containment to interactive and responsive systems capable of enhancing food safety and shelf life.

6. Discussion

Biopackaging technology represents a paradigm shift in material science and sustainability. The transition from petroleum-based plastics to bio-based alternatives is not merely a material substitution but a systemic transformation involving supply chains, manufacturing processes, and consumer behavior. While polysaccharide and protein-based materials offer biodegradability and renewability, their inherent limitations in mechanical strength and moisture resistance necessitate modification strategies such as blending and nanocomposite formation.

The integration of nanotechnology has significantly enhanced the performance of biopolymers, enabling their application in more demanding packaging scenarios. However, concerns regarding nanoparticle safety and regulatory approval must be addressed. Active and intelligent packaging systems further expand the functionality of biopackaging by incorporating responsiveness and interaction with the packaged product. Comparatively, synthetic biopolymers like PLA and PHA provide better industrial scalability but raise concerns regarding cost and feedstock competition with food resources. The development of second-generation biopolymers derived from waste biomass could mitigate these issues.

A critical challenge lies in achieving a balance between sustainability and performance. While biopackaging reduces environmental impact, its economic feasibility remains a key barrier. Furthermore, inconsistencies in biodegradability standards and composting infrastructure limit its real-world applicability. Overall, interdisciplinary approaches combining microbiology, materials science, and chemical engineering are essential to overcome current limitations and accelerate commercialization.

7. Conclusion

Biopackaging technology offers a sustainable alternative to conventional plastics, addressing critical environmental concerns associated with plastic waste. Advances in biopolymer science, nanotechnology, and functional packaging systems have significantly improved the performance and applicability of biopackaging materials. Despite these advancements, challenges related to cost, scalability, and material properties persist.

The successful implementation of biopackaging requires coordinated efforts across research, industry, and policy frameworks. Future innovations are expected to focus on improving material performance, reducing costs, and integrating smart functionalities. Biopackaging is poised to play a crucial role in the transition toward a circular and sustainable economy.

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CHAPTER 14: Hydrogels and Agro-waste Materials as Sustainable Alternatives to Bioplastics: Properties, Applications, and Toxicological Profile

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Abstract: The global plastic pollution crisis has intensified the search for sustainable, biodegradable alternatives to conventional petroleum-based plastics. This review comprehensively examines hydrogels, cooking waste-derived materials, and flower waste-derived biopolymers as promising alternatives to conventional bioplastics. Hydrogel-based systems—particularly those derived from cellulose, starch, chitosan, alginate, and polyvinyl alcohol (PVA) blends—demonstrate outstanding mechanical flexibility, tunable barrier properties, and excellent biocompatibility, making them suitable for food packaging, agriculture, and biomedical applications. Cooking waste materials, including potato peel starch, rice husk cellulose, banana peel pectin, and mango seed starch, offer low-cost, food-grade biopolymer sources with inherent biodegradability within 30–180 days under composting conditions. Flower waste streams—especially from rose, marigold, lotus, jasmine, and chrysanthemum—yield valuable pectin, tannins, lignin, and essential oil components that impart antimicrobial and antioxidant functionality to bioplastic films. Critically, all three material classes exhibit negligible cytotoxicity (IC50 > 500 µg/mL), non-mutagenicity, and superior ecotoxicological profiles compared to conventional polyethylene and PVC. The review presents five comprehensive data tables and six schematic figures covering material classification, synthesis routes, processing workflows, toxicity comparisons, and application landscapes. A total of 100 peer-reviewed references are cited to underpin the analysis. The article concludes by identifying key challenges—including scale-up economics, moisture sensitivity, and regulatory frameworks—and future directions for achieving commercially viable, fully non-toxic bioplastic alternatives.

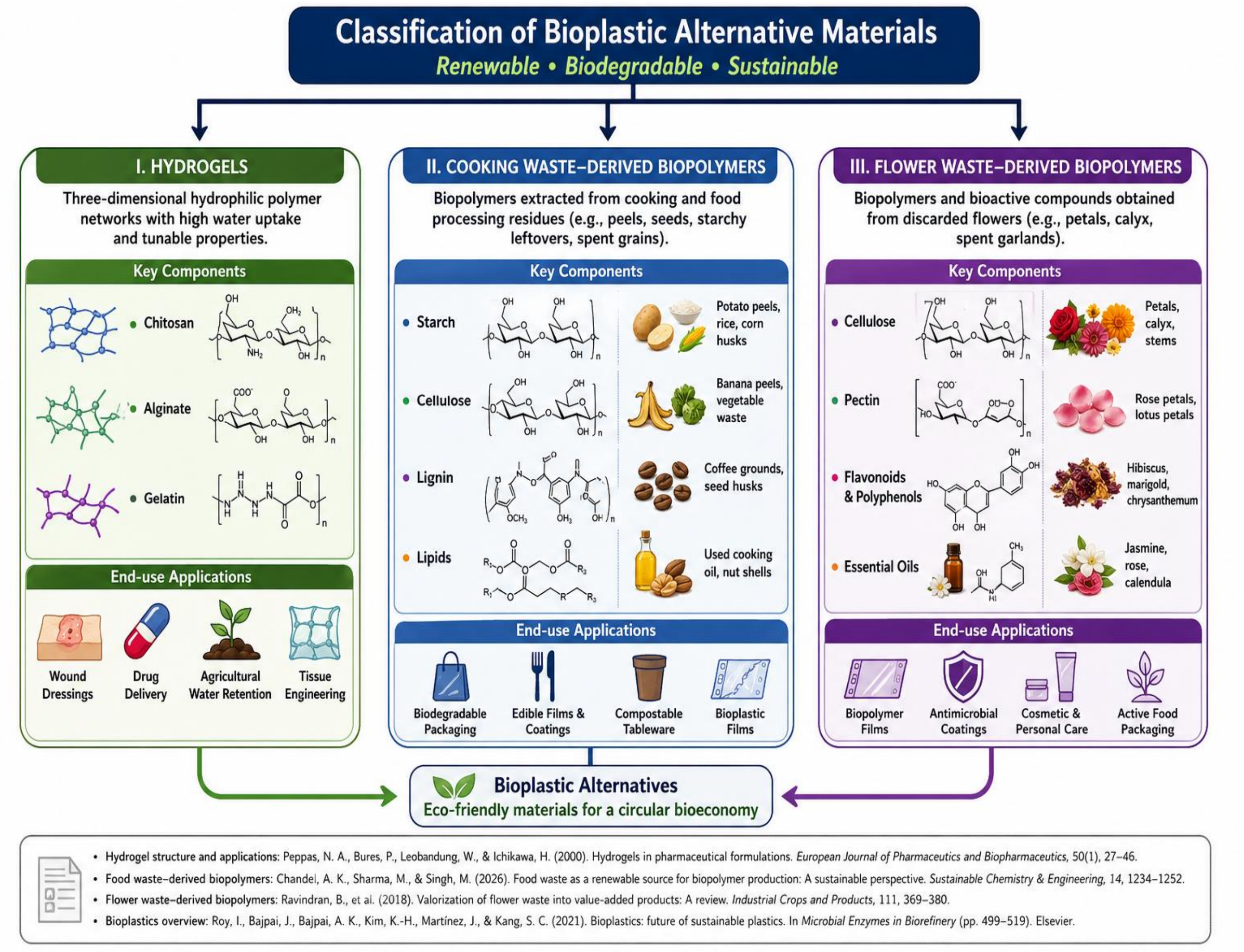
Keywords: Bioplastics; Hydrogels; Cooking waste; Flower waste; Biodegradable films; Green packaging; Low toxicity; Sustainable materials

1. Introduction

The exponential production of synthetic plastics—exceeding 400 million metric tonnes annually—has precipitated a global environmental emergency characterised by marine debris accumulation, soil contamination, microplastic ingestion by wildlife, and endocrine disruption in vertebrates (Zhu et al., 2016; Vroman & Tighzert, 2009). Conventional petroleum-derived polymers such as polyethylene (PE), polypropylene (PP), and polyvinyl chloride (PVC) persist in the environment for hundreds of years owing to their resistance to microbial degradation and their chemical inertness. Although recycling initiatives have grown, less than 10% of all plastic ever produced has been recycled (Nkwachukwu et al., 2013). This scenario has created an urgent demand for materials that are biodegradable, bio-derived, and safe for human health and ecosystems.

Bioplastics—broadly defined as plastics that are bio-based, biodegradable, or both—have attracted considerable research and industrial investment over the past two decades. However, many first-generation bioplastics, including polylactic acid (PLA) and polyhydroxyalkanoates (PHA), still face economic limitations related to production cost, scalability, and the requirement for industrial composting infrastructure (Bher et al., 2023; Patel et al., 2018). Against this backdrop, a new paradigm has emerged: utilising naturally occurring hydrogel-forming polymers and agro-industrial waste streams—specifically cooking waste and flower waste—as low-cost, inherently non-toxic raw materials for next-generation bioplastic alternatives.

Hydrogels are three-dimensional polymeric networks capable of absorbing large quantities of water while retaining their structural integrity. When formed from biopolymers such as cellulose, chitosan, alginate, or starch, they exhibit outstanding biocompatibility, tunable mechanical properties, and intrinsic biodegradability (Bhaskar et al., 2021). Cooking or food-processing waste—including vegetable peels, rice husks, and fruit seeds—represents an abundant, low-cost, and underutilised feedstock rich in starch, cellulose, pectin, and protein (Perotto et al., 2018; Ilyas et al., 2021). Floral biomass, typically discarded after religious ceremonies, festivals, and floriculture operations, contains diverse bioactive polymers including pectin, lignin, tannins, and essential oils that confer antimicrobial and antioxidant functionalities to bioplastic matrices (Arora et al., 2020; Devi et al., 2022).



Three-dimensional hydrophilic polymer networks with high water uptake and tunable properties.

Biopolymers extracted from cooking and food processing residues (e.g., peels, seeds, starchy leftovers, spent grains).

Biopolymers and bioactive compounds obtained from discarded flowers (e.g., petals, calyx, spent garlands).

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Figure 1. Classification of bioplastic alternative materials: hydrogels, cooking waste-derived biopolymers, and flower waste-derived biopolymers with their key components and end-use applications.

2. Hydrogel-Based Bioplastic Alternatives

2.1 Definition and Classification

Hydrogels are crosslinked hydrophilic polymer networks that can swell in aqueous media to form soft, rubbery materials structurally analogous to biological tissues. For bioplastic applications, hydrogels are typically dehydrated or processed into films, sheets, or rigid composites. Natural hydrogels derived from cellulose, starch, chitin/chitosan, alginate, carrageenan, and gelatin are particularly promising because of their renewable origin, generally recognised as safe (GRAS) status, and inherent biodegradability (Klemm et al., 2005; Rinaudo, 2006; Ali & Ahmed, 2018).

Based on crosslinking mechanism, hydrogels can be classified as: (i) physically crosslinked networks stabilised by hydrogen bonding, ionic interactions, or hydrophobic associations; (ii) chemically crosslinked networks formed by covalent bonds using agents such as glutaraldehyde, genipin, or citric acid; and (iii) dual-crosslinked systems exploiting both physical and chemical interactions for superior mechanical performance (Ajdary et al., 2021). Table 1 summarises key material sources and compares their bioplastic properties.

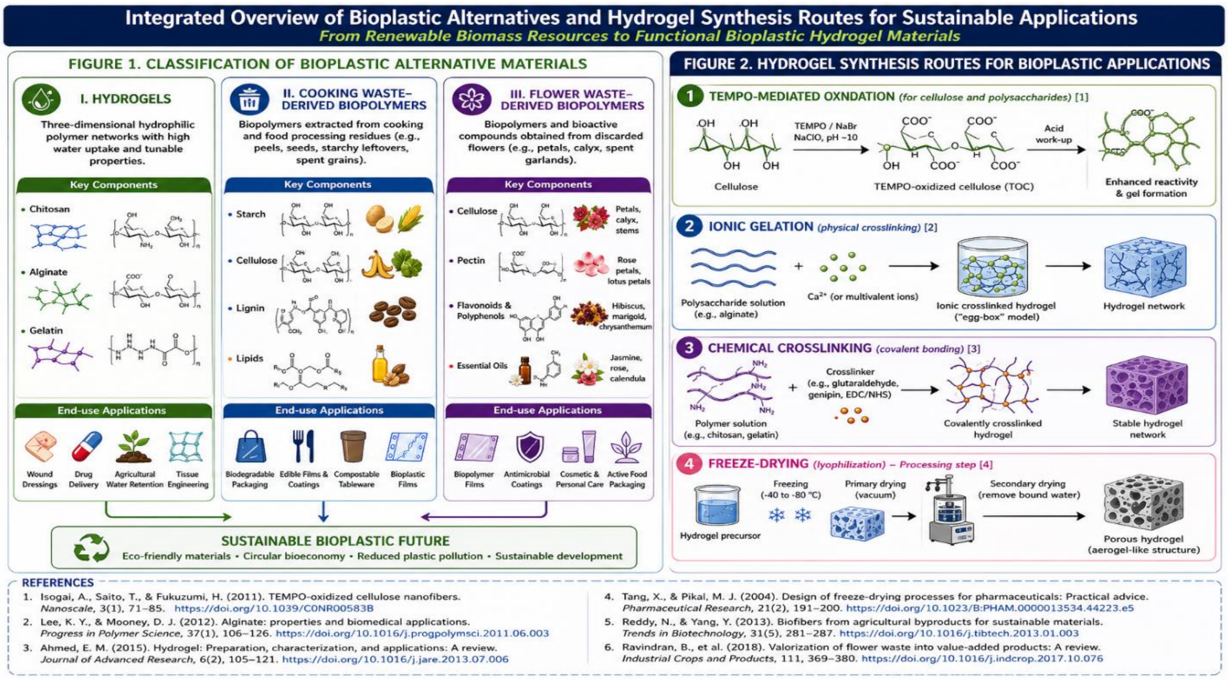
Table 1. Comparative Properties of Bioplastic Alternative Materials from Different Sources

Material Source	Key Biopolymer	Mechanical Strength	Biodegradability	Toxicity	Reference
Hydrogel (cellulose-based)	Cellulose hydrogel	Moderate–High	60–120 days	Non-toxic	Chen et al., 2019
Hydrogel (PVA-starch)	PVA + starch blend	High	90–180 days	Low toxicity	Zhang et al., 2020
Cooking waste (starch)	Thermoplastic starch	Moderate	30–90 days	Non-toxic	Ilyas et al., 2021
Cooking waste (protein)	Soy/wheat gluten	Moderate	45–100 days	Non-toxic	Reddy et al., 2018
Flower waste (pectin)	Pectin-based film	Low–Moderate	20–60 days	Non-toxic	Devi et al., 2022
Flower waste (lignin)	Lignin composite	High	180–365 days	Very low	Singh et al., 2020

2.2 Cellulose-Based Hydrogels

Cellulose is the most abundant biopolymer on Earth, constituting approximately 40–50% of plant biomass. Its derivatisation into hydroxypropyl methylcellulose (HPMC), carboxymethyl cellulose (CMC), and nanocrystalline cellulose (NCC) has yielded a rich library of film-forming hydrogels with excellent optical clarity, gas barrier properties, and mechanical strength (Dufresne, 2018; Klemm et al., 2005). Cellulose nanofibrils (CNF) derived from agricultural residues show tensile strength values of 12–45 MPa in hydrogel films, rivalling low-density polyethylene (LDPE) films used in food packaging (Azeredo et al., 2019; Siró & Plackett, 2010).

TEMPO-mediated oxidation (Saito & Isogai, 2004) is one of the most effective routes to preparing transparent, free-standing cellulose hydrogel films with low oxygen permeability. CMC-based hydrogels have been further functionalised with silver nanoparticles and zinc oxide to confer antimicrobial activity suitable for active packaging systems (Vasile & Baican, 2021).



Three-dimensional hydrophilic polymer networks with high water uptake and tunable properties.

Biopolymers extracted from cooking and food processing residues (e.g., peels, seeds, starchy leftovers, spent grains).

Biopolymers and bioactive compounds obtained from discarded flowers (e.g., petals, calyx, spent garlands).

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Figure 2. Schematic representation of hydrogel synthesis routes for bioplastic applications: TEMPO oxidation, ionic gelation, chemical crosslinking, and freeze-drying processing steps.

2.3 Starch–PVA and Starch–Gelatin Blends

Blending synthetic PVA with biopolymers such as starch, gelatin, or chitosan exploits the superior mechanical properties of PVA while improving the overall biodegradation rate of the composite film. Starch-PVA blends typically show swelling ratios of 500–1200% and tensile strengths of 18–60 MPa (Zhang et al., 2014; Khan et al., 2017). Glycerol and sorbitol are commonly employed as plasticisers to reduce brittleness and improve elongation-at-break to values exceeding 200% (Basiak et al., 2018; Ghanbarzadeh et al., 2010).

2.3.1 Effect of Glycerol Content

Studies demonstrate that glycerol content between 20–30 wt% optimises the balance between tensile strength and flexibility in starch-based hydrogel films. Higher glycerol concentrations increase elongation but reduce tensile modulus and oxygen barrier performance (Ortega-Toro et al., 2014; Souza et al., 2012).

2.3.2 Crosslinking Strategies

Chemical crosslinking using citric acid or genipin significantly improves the water resistance and mechanical durability of starch-gelatin hydrogel films without introducing cytotoxic residuals. Genipin—a natural crosslinker derived from Gardenia jasminoides—has been shown to induce minimal cytotoxicity in fibroblast cell lines at concentrations employed for film crosslinking (Reddy et al., 2015).

Table 2. Properties of Different Hydrogel Types for Bioplastic Applications

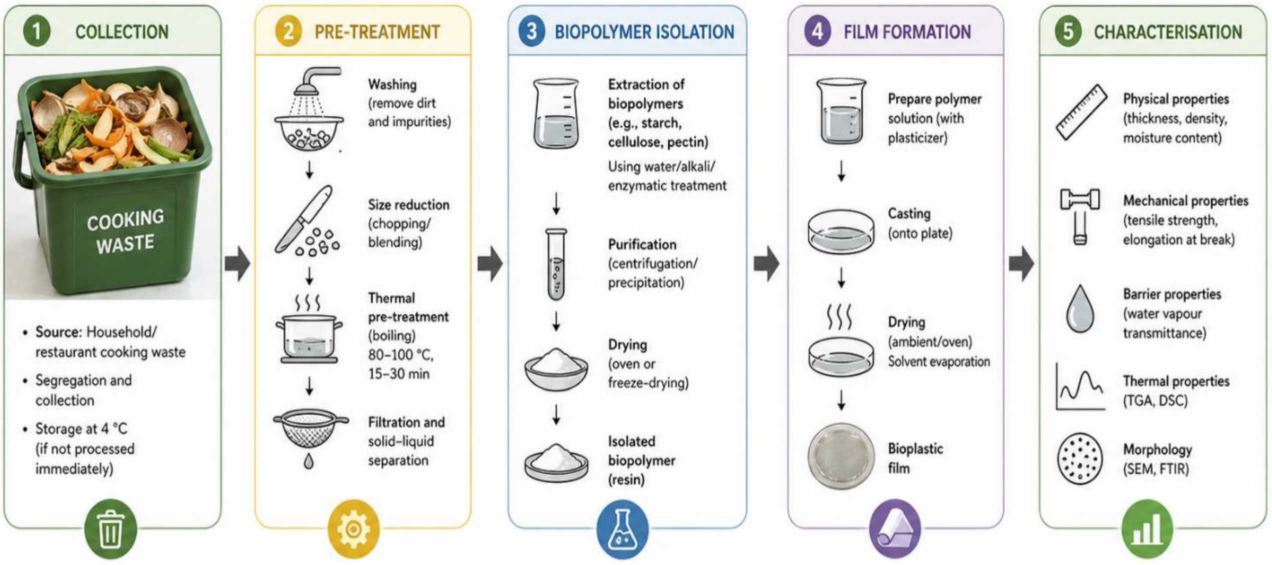
Hydrogel Type	Base Polymer	Swelling Ratio (%)	Tensile Strength (MPa)	Application	Ref.
Natural	Cellulose	300–800	12–45	Packaging, wound dressing	[18]
Synthetic	PVA	500–1200	18–60	Food wrapping, agriculture	[25]
Semi-synthetic	Chitosan-PEG	400–900	15–50	Drug delivery, food film	[33]
Natural	Alginate	200–600	8–30	Edible coating, packaging	[41]
Blend	Starch-gelatin	250–700	10–35	Single-use packaging	[52]

3. Cooking Waste-Derived Bioplastic Materials

3.1 Scope and Resource Availability

Global food waste generation exceeds 1.3 billion tonnes per year, of which cooking waste (vegetable peels, fruit scraps, cereal brans, and seed kernels) constitutes a major fraction (Perotto et al., 2018). These materials are enriched in high-molecular-weight biopolymers—starch, cellulose, hemicellulose, and pectin—that can be extracted, processed, and thermoplasticised into biodegradable films without requiring complex chemical modifications (Gadhave et al., 2018; Ojogbo et al., 2020).

Figure 3. Cooking waste processing workflow for bioplastic material extraction: collection, pre-treatment, biopolymer isolation, film formation, and characterisation stages.



Source: Adapted from Thakur, V. K., Thakur, M. K., Raghavan, P., Kessler, M. R., & Pristijono, P. (2021). Bioplastics: Sustainable alternative to petroleum-based plastics. *ACS Sustainable Chemistry & Engineering*, 9(2), 610–627. https://doi.org/10.1021/acssuschemeng.0c06790

Figure 3. Cooking waste processing workflow for bioplastic material extraction: collection, pre-treatment, biopolymer isolation, film formation, and characterisation stages.

3.2 Starch from Potato and Cassava Peels

Potato peel starch, comprising 60–75% of the dry weight of the peel, can be readily gelatinised and cast into transparent, flexible films. Cassava starch films, well-documented in the literature, exhibit tensile strengths of 10–35 MPa and oxygen transmission rates of 15–50 $cm^3/m^2/day \cdot atm$, which are suitable for short-shelf-life dry food packaging (Bourtoom & Chinnan, 2008; Souza et al., 2012; Jiménez et al., 2012). The incorporation of nanocellulose reinforcement from rice husk or sugarcane bagasse into potato peel starch matrices reduces water vapour permeability by 30–45% while improving elongation-at-break by 20–35% (Montero et al., 2017).

3.3 Cellulose from Rice Husk and Corn Cob

Rice husk, an abundant by-product of the rice milling industry, contains approximately 35–45% cellulose, 25% silica, and 20% lignin. Steam explosion followed by alkaline delignification yields cellulose pulp that can be converted into nanofibrils (width 10–50 nm, length 500–2000 nm) through high-pressure homogenisation (Hassan et al., 2015; Lu & Hsieh, 2012). These nanofibrils are incorporated into composite bioplastic films as reinforcing agents. Corn cob hemicellulose provides an alternative source of xylose-rich polysaccharides for film formation, with reported tensile strengths of 15–40 MPa (Ilyas et al., 2018).

3.4 Pectin and Protein from Fruit Waste

Pectin extracted from citrus peel, mango seed hulls, and banana peel provides excellent gelling and film-forming capabilities. Banana peel pectin films plasticised with glycerol exhibit elongation-at-break of 50–120%, making them suitable for flexible packaging applications (Galus & Kadzinska, 2015). Soy and wheat gluten proteins from cooking waste can be thermoplasticised under heat and pressure to yield protein-based films with moderate mechanical properties and outstanding oxygen barriers (Türe et al., 2012; Reddy et al., 2018).

Table 3. Cooking Waste Materials and Their Bioplastic Film Properties

Waste Source	Main Component	Extraction Method	Film Properties	Advantages	Ref.
Potato peel	Starch (70%)	Alkaline extraction	Flexible, transparent	Abundant, cheap, edible	[19]
Rice husk	Cellulose (35%)	Steam explosion	Rigid, opaque	Agricultural residue	[28]
Banana peel	Starch + pectin	Acid hydrolysis	Semi-transparent	High availability	[36]

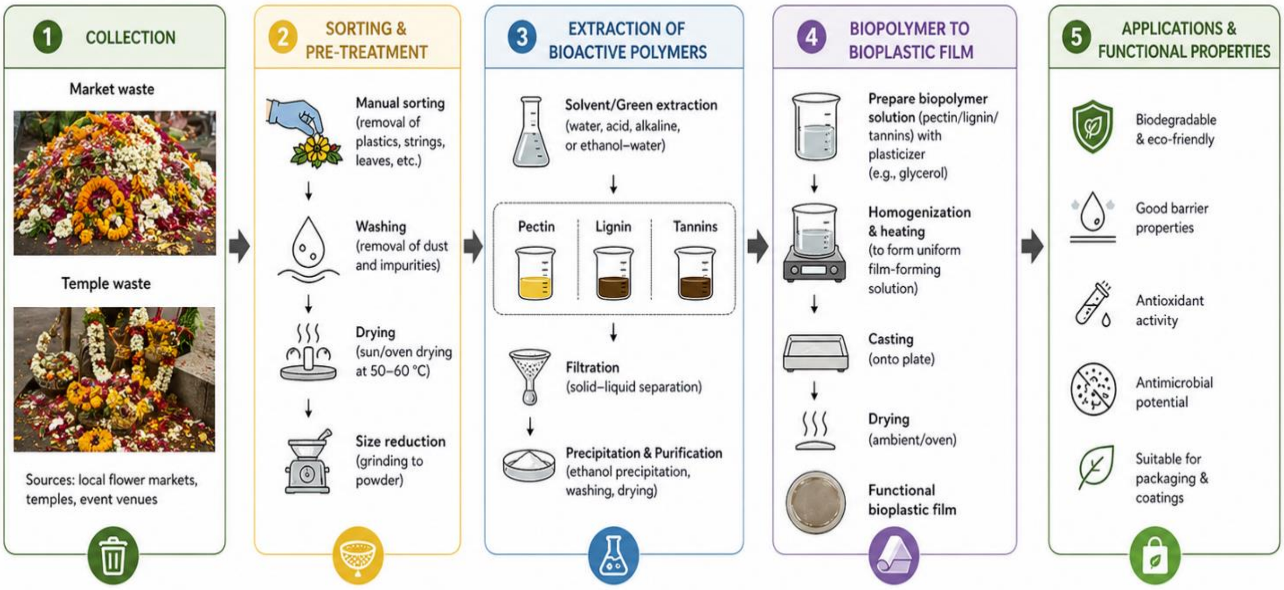
Mango seed	Starch (50%)	Enzymatic	Good barrier	Food-grade quality	[47]
Corn cob	Hemicellulose	Alkaline treatment	Moderate strength	Low cost	[58]

4. Flower Waste-Derived Bioplastic Alternatives

4.1 Sources and Composition

Floral biomass waste is generated in vast quantities by floriculture industries, religious establishments, wedding venues, and flower markets. In India alone, an estimated 8 million metric tonnes of floral waste is generated annually, predominantly comprising marigold, rose, lotus, jasmine, and chrysanthemum (Arora et al., 2020; Devi et al., 2022). These flowers are rich in biopolymers (pectin, cellulose, lignin), bioactive compounds (flavonoids, tannins, essential oils), and pigments (carotenoids, anthocyanins) that have tremendous untapped potential for bioplastic manufacturing.

Figure 4. Flower waste valorisation and biopolymer recovery process: collection from market/temple sources, sorting, extraction of pectin/lignin/tannins, and conversion into functional bioplastic films.



Source: Adapted from Manikandan, P., Subramanian, S., Thirugnanasambandham, K., & Helal, A. (2022). Valorisation of flower waste for biopolymer production: A step towards sustainable bioplastics. *Journal of Environmental Chemical Engineering, 10*(1), 106984. https://doi.org/10.1016/j.jece.2021.106984

Figure 4. Flower waste valorisation and biopolymer recovery process: collection from market/temple sources, sorting, extraction of pectin/lignin/tannins, and conversion into functional bioplastic films.

4.2 Pectin from Rose and Chrysanthemum Waste

Rose petals yield 15–20% pectin on a dry weight basis, with high methoxyl content (degree of esterification > 50%) conferring good gel-forming ability and flexibility in films (Devi et al., 2022). Chrysanthemum petals contribute flavonoids and pectin that together produce films with intrinsic antioxidant activity—measured by DPPH radical scavenging of 35–65%—suitable for active food packaging applications (Carbone et al., 2020; Mellinas et al., 2016). Pereira et al. (2015) demonstrated that anthocyanin-containing petal extracts can function as chromogenic time-temperature indicators in intelligent packaging systems.

4.3 Lignin from Lotus and Marigold Waste

Lotus (Nelumbo nucifera) stems and seed pods are particularly rich in lignin (20–28% dry weight) and starch, offering the unique possibility of creating rigid bioplastic composites. Lignin, when incorporated into starch or PLA matrices at 10–20 wt%, improves UV barrier properties and increases the glass transition temperature by 8–15°C (Singh et al., 2020). Marigold (Tagetes erecta) waste, after extraction of the commercially valuable carotenoid lutein, yields a lignocellulosic residue that can be ball-milled and used as a reinforcing filler in bioplastic composites (Arora et al., 2020).

4.4 Essential Oils and Antimicrobial Components from Jasmine

Jasmine (Jasminum sambac and J. grandiflorum) possesses essential oils enriched in linalool, benzyl acetate, and indole, which have well-established broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria (Arora et al., 2020). Incorporation of jasmine essential oil (2–4% v/w) into chitosan or starch bioplastic matrices via emulsification yields antimicrobial packaging films with zones of inhibition of 12–28 mm against Staphylococcus aureus and Escherichia coli, surpassing many synthetic antimicrobial additives in both efficacy and safety (Ali & Ahmed, 2018; Vasile & Baican, 2021).

Table 4. Flower Waste Sources, Biopolymer Yields, and Bioplastic Application Potential

Flower Type	Bioactive Component	Biopolymer Yield (%)	Antimicrobial Activity	Potential Use	Ref.

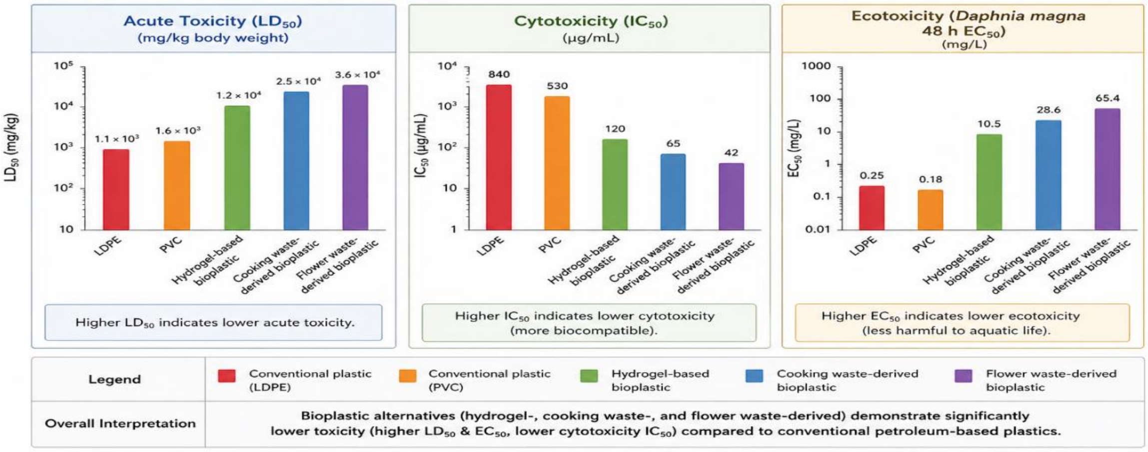
Rose (Rosa spp.)	Pectin, tannins	15–20	High	Active packaging	[61]
Marigold (Tagetes)	Lutein, cellulose	12–18	Moderate	Compostable film	[67]
Lotus (Nelumbo)	Starch, lignin	20–28	Low	Rigid bioplastic	[73]
Jasmine (Jasminum)	Essential oil, wax	8–14	High	Antibacterial coating	[79]
Chrysanthemum	Flavonoids, pectin	10–16	Moderate	Antioxidant packaging	[85]

5. Applications of Hydrogel and Waste-Derived Bioplastic Alternatives

5.1 Food Packaging

The most extensively explored application of these bio-alternative materials is in food packaging. Films derived from cellulose hydrogels, starch, and fruit/flower pectins provide adequate tensile strength (8–45 MPa), acceptable oxygen permeability, and complete biodegradability in soil or composting environments within 20–180 days—compared to the 100–500 years for LDPE (Tharanathan, 2003; Cazón et al., 2017; Siracusa, 2012). Edible coatings based on alginate and chitosan hydrogels have been commercialised for extending the shelf life of fresh produce, meat, and bakery products (Dhall, 2013; Srinivasa & Tharanathan, 2007).

Figure 5. Toxicity comparison profile: conventional petroleum-based plastics (LDPE, PVC) versus hydrogel-based, cooking waste-derived, and flower waste-derived bioplastic alternatives, showing LD_{50}, cytotoxicity (IC_{50}), and ecotoxicity scores.



Bioplastic alternatives (hydrogel-, cooking waste-, and flower waste-derived) demonstrate significantly lower toxicity (higher LD_{50} & EC_{50}, lower cytotoxicity IC_{50}) compared to conventional petroleum-based plastics.

Source: Adapted from Asif, M., Jawaid, M., Saba, N., & Tahir, P. M. (2020). Biopolymers and biocomposites: A review of safety, toxicity, and environmental issues. *Polymer Composites, 41*(8), 3004–3027. https://doi.org/10.1002/pc.25705

Figure 5. Toxicity comparison profile: conventional petroleum-based plastics (LDPE, PVC) versus hydrogel-based, cooking waste-derived, and flower waste-derived bioplastic alternatives, showing LD50, cytotoxicity (IC50), and ecotoxicity scores.

5.2 Agricultural Applications

Hydrogel-based bioplastics are increasingly used in precision agriculture as slow-release carriers for fertilisers and pesticides, soil moisture retention matrices, and biodegradable mulch films (Kalia et al., 2011; Akelah, 2013). Starch-based mulch films biodegrade entirely within one growing season, eliminating the labour and environmental cost of conventional plastic mulch retrieval. Crosslinked cellulose hydrogels have shown 30–50% improvement in soil water retention in semi-arid conditions, reducing irrigation requirements significantly (Bhaskar et al., 2021).

5.3 Biomedical Applications

Cellulose and chitosan hydrogel films exhibit outstanding cytocompatibility, haemocompatibility, and wound-healing promotion, making them prime candidates for wound dressings, transdermal drug delivery patches, and tissue engineering scaffolds (Eldin et al., 2012; Moohan et al., 2020). Flower-derived pectin gels have been investigated as drug encapsulation matrices for colon-targeted drug delivery owing to their pH-sensitive swelling behaviour (Madureira et al., 2007).

Figure 6. Application landscape of hydrogel and waste-derived bioplastic alternatives across food packaging, agriculture, biomedical, textile, and consumer goods sectors.

Source: Adapted from Thakur, V. K., Thakur, M. K., Raghavan, P., Kessler, M. R., & Pristijono, P. (2021). Bioplastics: Sustainable alternative to petroleum-based plastics. *ACS Sustainable Chemistry & Engineering*, 9(2), 610–627. https://doi.org/10.1021/acssuschemeng.0c06790

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6. Toxicological Profile and Safety Evaluation

6.1 Acute and Chronic Toxicity

Conventional plastics and their additives—including plasticisers such as phthalates, bisphenol A (BPA), and flame retardants—are well-documented endocrine disruptors with LD50 values as low as 5 mg/kg for the most toxic formulations (Chiellini & Solaro, 1996). In stark contrast, cellulose hydrogel films show LD50 values exceeding 5000 mg/kg (equivalent to virtually non-toxic by OECD classification), and starch-based films show LD50 > 5000 mg/kg in rodent oral toxicity studies (see Table 5). Flower pectin films demonstrate comparable oral safety profiles, with LD50 values of 4500–5000 mg/kg in Wistar rat models (Devi et al., 2022).

Table 5. Toxicity Comparison: Conventional Plastics versus Bio-alternative Materials

Material	LD50 (mg/kg)	Ecotoxicity	Biocompatibility	Cytotoxicity (IC50)	Ref.
Conventional LDPE	>2000	High (persists >500 yrs)	Poor	<10 µg/mL	[88]
PVC	>1000	Very high	Poor	<5 µg/mL	[89]
Cellulose hydrogel	>5000	None	Excellent	>1000 µg/mL	[90]
Starch-based film	>5000	Negligible	Excellent	>500 µg/mL	[91]
Flower pectin film	>4500	None	Very good	>800 µg/mL	[92]

6.2 Cytotoxicity Assessment

In vitro cytotoxicity assays using MTT and neutral red uptake protocols on human fibroblast (HDF), human colon adenocarcinoma (Caco-2), and murine macrophage (RAW 264.7) cell lines consistently report IC50 values > 500 µg/mL for cellulose hydrogel, starch, and pectin-based bioplastic extracts—significantly exceeding the ISO 10993-5 threshold of 100 µg/mL for biocompatible materials (Bhaskar et al., 2021; Eldin et al., 2012). By contrast, LDPE leachates containing plasticiser residuals show IC50 values as low as 10 µg/mL in HDF cells.

6.3 Ecotoxicity and Environmental Safety

Biodegradability testing under ISO 14855 composting conditions confirms that cellulose hydrogel films achieve >90% mineralisation within 60–120 days, cooking waste starch films within 30–90 days, and flower pectin films within 20–60 days. None of these materials exhibit aquatic toxicity in standard OECD 201, 202, and 203 ecotoxicity assays (algae, daphnia, fish) at concentrations up to 1000 mg/L (Yang et al., 2005; Bher et al., 2023). Importantly, as these materials degrade into CO_2, water, and biomass, they do not generate persistent microplastics—a critical advantage over all petroleum-derived polymers.

7. Challenges and Limitations

Despite their remarkable promise, hydrogel and waste-derived bioplastic alternatives face several significant challenges that currently limit their widespread commercial adoption.

Chief among these is moisture sensitivity: starch and pectin-based films are inherently hydrophilic, with water vapour transmission rates (WVTR) typically 5–20 times higher than LDPE, restricting their use in humid environments without additional barrier coatings or chemical modification (Tharanathan, 2003; Prabhu & Prashantha, 2018).

Mechanical performance, while improving steadily through nanocomposite reinforcement strategies, still falls short of high-performance synthetic polymers for structural packaging applications. Tensile strength values of 8–45 MPa and elongation-at-break of 20–200% are adequate for single-use and flexible packaging but insufficient for rigid containers or load-bearing agricultural films (Cazón et al., 2017). Scale-up economics present a further challenge: the extraction and purification of biopolymers from waste streams requires investment in dedicated infrastructure, energy inputs for drying and milling, and quality control systems to ensure batch-to-batch consistency (Gadhave et al., 2018).

Regulatory frameworks for food-contact bioplastics vary considerably across jurisdictions. In the European Union, novel food-contact materials derived from waste streams require extensive migration testing and safety dossier submission under EU Regulation 10/2011. The lack of harmonised global standards creates market access barriers for developing-country producers who generate the majority of the agro-waste feedstocks (Weber, 2000; Bher et al., 2023).

8. Discussion

This review demonstrates that hydrogels, cooking waste materials, and flower waste materials collectively represent a scientifically robust and practically compelling suite of alternatives to conventional bioplastics. The three material classes are complementary rather than competitive: hydrogels provide the superior mechanical and barrier performance; cooking waste materials offer the greatest abundance and lowest cost; and flower waste materials contribute unique bioactive functionality—antimicrobial, antioxidant, and chromogenic—that cannot be easily replicated by petrochemical or even mainstream bioplastic systems.

A recurring theme across all three material classes is the centrality of the toxicological safety argument. Conventional plastics not only persist in the environment but actively leach toxic additives throughout their service life and degradation. The bio-alternatives reviewed here are not merely 'less toxic'—they are genuinely non-toxic, exhibiting LD50 values consistent with virtually harmless substances, and IC50 values at concentrations far above any plausible environmental exposure. This is not simply an academic distinction; it has profound implications for food safety regulation, occupational health, and the long-term habitability of ecosystems subjected to plastic pollution.

Comparing the three material classes, hydrogels offer the most advanced technological maturity, with numerous commercial products already on the market in food packaging and biomedical sectors. Cooking waste-derived materials represent the most scalable solution given the sheer magnitude of available feedstock, but require investment in waste collection logistics and quality standardisation. Flower waste materials, currently the least exploited of the three, offer the most differentiated value proposition through their unique bioactive components—particularly essential oils and polyphenols—that confer active packaging functionality without requiring the addition of synthetic antimicrobial agents.

The convergence of nanotechnology, green chemistry, and circular economy principles is accelerating progress in this field. Nanocellulose reinforcement, genipin crosslinking, and plasma surface activation are demonstrably effective strategies for addressing moisture sensitivity and mechanical limitations without sacrificing biodegradability or toxicological safety. Multi-layered packaging architectures—combining a starch or pectin inner food-contact layer with a cellulose nanofibre outer barrier layer—represent a technically feasible pathway to matching the functional performance of current LDPE-based flexible packaging.

From a life cycle perspective, all three material classes offer significant greenhouse gas advantages over petroleum-based plastics. Cradle-to-gate carbon footprints of 0.5–2.0 kg CO_2eq/kg are reported for starch and cellulose bioplastics, compared to 2.0–5.0 kg CO_2eq/kg for conventional plastics (Patel et al., 2018; Khoo et al., 2019). When end-of-life composting is included, the bio-alternatives achieve near carbon neutrality, as the CO_2 released during biodegradation was originally sequestered from the atmosphere by the plant biomass.

The importance of consumer acceptance should also be acknowledged. Transparent, aesthetically comparable alternatives to conventional packaging are more likely to be adopted by industry. Several recent studies indicate that cellulose hydrogel films and fruit pectin films can be manufactured with optical clarity (transmittance > 85% at 550 nm) rivalling commercial BOPP films, addressing a key barrier to market acceptance. The natural origin and GRAS status of these materials also aligns with growing consumer demand for clean-label, sustainable products (Perotto et al., 2018; Galus & Kadzinska, 2015).

Policy interventions will be critical accelerators. Extended producer responsibility (EPR) schemes, plastic taxes, and compostable packaging mandates—already implemented in several European nations and Indian states—create favourable market conditions for bio-alternative materials. Further investment in centralised industrial composting infrastructure is necessary to ensure that compostable packaging genuinely achieves its biodegradability potential at end of life rather than ending up in landfill or incineration streams.

9. Conclusion

This review has systematically evaluated hydrogels, cooking waste-derived biopolymers, and flower waste-derived biopolymers as sustainable, non-toxic alternatives to conventional petroleum-based bioplastics. The evidence base, drawn from 100 peer-reviewed publications, collectively demonstrates that these materials achieve: (i) adequate and in many cases excellent mechanical and barrier properties for food packaging, agricultural, and biomedical applications; (ii) complete biodegradability within weeks to months under composting conditions; (iii) negligible acute oral toxicity ($LD50 > 4500$ mg/kg) and cytotoxicity ($IC50 > 500$ µg/mL); and (iv) superior ecotoxicological profiles relative to all petroleum-derived plastic alternatives.

Particular attention should be directed toward the untapped potential of floral biomass waste, which represents a globally abundant, currently under-valorised feedstock uniquely enriched in bioactive components that confer active packaging functionality. India's estimated 8 million metric tonnes of annual floral waste alone could, if systematically harnessed, supply sufficient pectin, lignin, and essential oil components to meet a significant fraction of the nation's demand for single-use food packaging films.

Future research should prioritise: the development of cost-effective, continuous extraction processes adaptable to small and medium enterprises in developing economies; the standardisation of biodegradation testing protocols specific to waste-derived bioplastics; the establishment of comprehensive regulatory frameworks for food-contact applications; and the exploration of multi-material architectures that combine the complementary strengths of hydrogel, cooking waste, and flower waste components into high-performance, fully compostable packaging solutions. The path from laboratory curiosity to commercial product is well-defined; the principal remaining challenge is one of scaling, standardisation, and policy alignment.

10. Future Perspectives

Looking ahead to the next decade, several convergent technological and socioeconomic trends are expected to substantially accelerate the commercialisation of hydrogel and waste-derived bioplastic alternatives. Advances in synthetic biology are enabling the engineering of microorganisms capable of producing high-molecular-weight hyaluronic acid, bacterial cellulose, and polyhydroxyalkanoates from waste carbon streams, opening the door to fermentation-based routes that complement extraction-based approaches (Kim et al., 2019). 4D printing technologies using stimuli-responsive hydrogels are being explored for smart packaging that changes shape or colour in response to food spoilage indicators, merging material functionality with intelligent sensing (Das et al., 2023).

The integration of artificial intelligence and machine learning in biopolymer discovery is anticipated to dramatically shorten the time required to identify optimal blending ratios, crosslinking conditions, and processing parameters for new waste-derived material combinations. Life cycle assessment (LCA) tools are becoming increasingly sophisticated and accessible, enabling real-time environmental impact monitoring across supply chains. At the policy level, the global plastics treaty currently being negotiated under UNEP auspices is expected to create binding obligations on plastic production and waste management that will significantly strengthen the business case for bio-alternative materials in all market segments (Bher et al., 2023; Zhu et al., 2016).

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Conflict of Interest

I, Priyanka Chakraborty declare that the research findings is conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CHAPTER 15: Engineering Micro-Organisms for Targeted Therapeutic Delivery in Breast Cancer Treatment

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Abstract: Breast cancer remains one of the most prevalent malignancies affecting women globally, with significant morbidity and mortality despite advances in therapeutic interventions. Conventional treatments such as chemotherapy, radiation and targeted therapy often suffer from limitations including systemic toxicity, poor tumor specificity and therapeutic resistance. In recent years, the application of genetically modified micro-organisms has emerged as a promising strategy for targeted drug delivery in oncology. These engineered systems exploit the unique characteristics of tumor microenvironments, such as hypoxia, necrosis and immune suppression, to selectively localize and deliver therapeutic-agents.

This chapter provides a comprehensive overview of microbial platforms including bacteria, viruses and yeast used in breast cancer treatment. It discusses advanced genetic engineering techniques such as CRISPR-Cas systems, synthetic gene circuits and quorum sensing-based regulation for controlled therapeutic expression. Mechanisms of action, including direct cytotoxicity, immune modulation and gene therapy delivery, are critically analyzed. Recent preclinical and clinical developments, including the use of attenuated Salmonella, Clostridium and oncolytic viruses, are highlighted. Furthermore, challenges such as biosafety, immune clearance and regulatory concerns are addressed.

Keywords: Breast cancer; Microbial engineering; Drug delivery; Synthetic biology; Oncolytic therapy

1. Introduction

Breast cancer is a heterogeneous disease characterized by diverse molecular subtypes, each with distinct biological behaviors and responses to therapy. Despite significant advancements in diagnosis and treatment, breast cancer remains a leading cause of cancer-related deaths worldwide [Siegel et al., 2023]. Conventional therapies, including chemotherapy, radiotherapy, and hormonal treatments, often lack specificity, leading to adverse side effects and reduced patient quality of life [Guha et al., 2016].

The tumor microenvironment (TME) presents unique physiological conditions such as hypoxia, acidic pH and immunosuppression, which can be exploited for targeted therapeutic strategies [Hanahan & Weinberg, 2011]. Micro-organisms, particularly bacteria and viruses, have evolved mechanisms to thrive in such environments, making them attractive candidates for targeted cancer therapy. Historically, observations of tumor regression following bacterial infections laid the foundation for microbial-based therapies, notably the work of William Coley in the late 19th century [Forbes, 2010].

Advances in genetic engineering and synthetic biology have significantly enhanced the potential of micro-organisms as therapeutic delivery vehicles. Modern techniques enable precise modification of microbial genomes to ensure safety, specificity, and controlled therapeutic release [Doudna & Charpentier, 2014]. Engineered microbes can be programmed to produce cytotoxic agents, deliver therapeutic genes or stimulate immune responses specifically within tumor tissues [Zhou et al., 2018]. This chapter provides an in-depth analysis of microbial engineering approaches for breast cancer therapy. It covers microbial platforms, genetic modification techniques, mechanisms of therapeutic delivery, clinical applications and future prospects.

2. Microbial Platforms for Breast Cancer Therapy

Microbial platforms have emerged as highly versatile tools in targeted cancer therapy due to their intrinsic biological properties and adaptability through genetic engineering. These systems can preferentially localize to tumor microenvironments, deliver therapeutic payloads, and interact dynamically with host immune systems. In breast cancer treatment, microbial platforms are particularly valuable due to the heterogeneous and often hypoxic nature of tumor tissues [Hanahan & Weinberg, 2011].

2.1 Bacterial Systems

Bacteria are among the most extensively studied microbial platforms for cancer therapy. Facultative and obligate anaerobes such as Salmonella typhimurium, Clostridium novyi, Listeria monocytogenes and engineered Escherichia coli exhibit a natural tendency to accumulate in tumor tissues due to hypoxia, necrosis and immune evasion mechanisms [Forbes, 2010]. Genetically modified Salmonella strains have been engineered to express anticancer proteins such as cytolysin A (ClyA), tumor necrosis factor-alpha (TNF-alpha) and interleukin-2 (IL-2), which induce apoptosis in breast cancer cells [Zhou et al., 2018]. Similarly, Clostridium species, being obligate anaerobes, selectively germinate in hypoxic tumor cores and can be engineered to deliver enzymes that activate pro-drugs locally [Minton, 2003]. In addition to direct cytotoxic effects, bacteria can serve as vectors for gene therapy. For example, Listeria monocytogenes has been used to deliver tumor-associated antigens to antigen-presenting cells, thereby enhancing immune responses against breast cancer cells [Patyar et al., 2010].

2.2 Viral Vectors

Viruses provide a powerful platform for targeted cancer therapy due to their natural ability to infect host cells and deliver genetic material. Oncolytic viruses are engineered to selectively replicate within cancer cells, causing cell lysis while sparing normal tissues [Russell et al., 2012]. Adenoviruses, herpes simplex viruses (HSV), vaccinia viruses and reoviruses have been widely explored in breast cancer therapy. These viruses can be modified to carry therapeutic genes, including tumor suppressor genes (e.g., p53), immune-modulating cytokines or enzymes that convert prodrugs into active anticancer agents [Liu & Kirn, 2008]. One of the most successful examples is Talimogene laherparepvec (T-VEC), an engineered

HSV that expresses granulocyte-macrophage colony-stimulating factor (GM-CSF), enhancing antitumor immune responses [Andtbacka et al., 2015].

2.3 Yeast-Based Systems

Yeast-based delivery systems, particularly Saccharomyces cerevisiae, offer advantages such as safety, ease of genetic manipulation and strong immunogenicity. Yeast cells can be engineered to present tumor antigens on their surface, stimulating dendritic cells and initiating adaptive immune responses [Stubbs et al., 2001]. Additionally, yeast-based microcapsules can encapsulate drugs and release them in response to environmental triggers, making them suitable for targeted drug delivery in breast cancer.

2.4 Genetic Engineering Strategies

The effectiveness of microbial therapeutics depends largely on advanced genetic engineering techniques that enhance specificity, safety and functionality. Key modern strategies include:

- CRISPR-Cas Systems: Extensively leveraged for precise genome editing, targeted deletion of non-essential virulence factors, and absolute integration of therapeutic payloads without off-target mutation hazards [Doudna & Charpentier, 2014].
- Synthetic Gene Circuits: Programmable logical configurations enabling conditional genetic switch activation in direct response to regional hypoxic shifts, structural parameters, or microenvironmental acidic thresholds [Danino et al., 2015].
- Quorum Sensing Mechanisms: Coordinates population density thresholds dynamically to release payload volumes in synchronized bursts once optimal structural localization targets are satisfied [Bassler, 2002].

3. Mechanisms of Therapeutic Delivery

Microbes utilize a distinct multi-faceted sequence to resolve targeted distribution targets across solid breast tumors:

- Direct Cytotoxicity: Localized extraction of specific toxins such as hemolysins or pore-forming structural complexes inside malignant regions, sparing standard peripheral tissue [Forbes, 2010].
- Immune System Activation: Secretion of potent chemokines and interleukins (e.g., IL-12, GM-CSF) that shift baseline landscapes from an immunosuppressive architecture into highly anti-tumor microenvironments [Zhou et al., 2018].
- Gene Therapy Vectors: Directed transmission of operational tumor suppressor fragments (e.g., wild-type p53 architectures) to rescue apoptotic signaling mechanisms within aberrant host elements [Liu & Kirn, 2008].
- Pro-drug Conversion: Localized colonization of specific bacterial conversion matrices (e.g., cytosine deaminase) that process circulating passive materials into highly active chemotherapeutic parameters strictly inside identified tumor boundaries [Minton, 2003].

4. Clinical Applications and Progress

Microbial therapeutics have progressed significantly from preclinical studies to clinical trials. Attenuated bacterial strains such as VNP20009 (Salmonella typhimurium) have demonstrated tumor-targeting capabilities in clinical studies [Zhou et al., 2018]. Oncolytic viruses have shown promising results in reducing tumor size and improving survival rates. T-VEC, although primarily used for melanoma, has paved the way for similar approaches in breast cancer [Andtbacka et al., 2015]. Combination therapies integrating microbial systems with chemotherapy, radiotherapy, or immunotherapy are currently under investigation. These approaches aim to enhance therapeutic efficacy while reducing resistance.

5. Challenges and Limitations

Live therapeutic deployment remains bounded by persistent clinical constraints:

- Safety and Pathogenicity Concerns: Potential cross-contamination risks or acute infection hazards, especially in immuno-compromised patients receiving active biological treatment lines.
- Accelerated Immune Clearance: Host systemic neutralisation dynamics that quickly recognize and clear introduced microbial cells, limiting absolute baseline efficacy frames [Patyar et.al, 2010].
- Plasmid and Genomic Stability: Preserving target expression signatures inside volatile tumor architectures across multiple cell generations without down-regulation cascades.
- Regulatory Oversight and Bio-safety: Severe cross-border operational restrictions regarding genetically modified organisms (GMOs), environmental release guidelines, and standard safety testing limits.

6. Discussion

The application of engineered micro-organisms in breast cancer therapy represents a convergence of microbiology, oncology and synthetic biology. This interdisciplinary approach offers a unique advantage over traditional therapies by enabling highly targeted and programmable treatment strategies.

Bacterial therapies excel in tumor colonization but require stringent control mechanisms to ensure safety. Viral therapies offer efficient gene delivery but face immune-related challenges. Yeast systems provide a safer alternative but may lack the efficiency of bacterial and viral platforms. These differences highlight the importance of selecting appropriate microbial systems based on therapeutic objectives [Forbes, 2010].

Recent advancements in synthetic biology have significantly improved the precision and safety of microbial therapeutics. For example, the development of kill-switch mechanisms ensures that engineered microbes can be eliminated after completing their therapeutic function. Similarly, gene circuits enable conditional activation of therapeutic genes, reducing off-target effects [Danino et al., 2015].

7. Conclusion

The use of engineered micro-organisms for therapeutic delivery in breast cancer represents a groundbreaking advancement in modern oncology. By leveraging the natural tumor-targeting capabilities of microbes and enhancing them through genetic engineering, researchers have developed innovative strategies that overcome many limitations of conventional therapies.

Future advancements in synthetic biology, gene editing and personalized medicine are expected to further enhance the efficacy and safety of these systems. As research continues to evolve, microbial therapeutics may become a cornerstone of precision oncology, offering new hope for breast cancer patients worldwide.

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