Microbial Menace and Miracle: The Double-Edged Sword of Infection and Immunity

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Contents

A Comparative Analysis of Bioremediation Technique for Treating Industrial Wastewater

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ABSTRACT

The rise in heavy metal concentrations in wastewater can be attributed to industrial and developmental activities, coupled with inadequate treatment before disposal, leading to considerable environmental issues. Lead (Pb) is a highly toxic heavy metal commonly used across various industries, including paint, batteries, electroplating, tanneries, paper and pulp mills, textile mills, smelting, and mining. As a result, significant amounts of lead are produced during the disposal of wastewater from these companies. The potential bioavailability of lead poses a significant health risk to children, resulting in cognitive impairment. The removal of this heavy metal from industrial effluents is essential before their release into the environment and natural land or water. Lead remediation in industrial wastewater is carried out using a range of conventional techniques. The main limitations of these methods are their limited effectiveness and lack of adequate technical knowledge. The widespread acceptance and cost-effectiveness of microbial lead treatment for industrial wastewater have recently surfaced as a practical alternative. Utilizing biotechnological techniques for the recovery of metals from effluents rich in heavy metals released into ponds is advisable. Microorganisms can effectively remove heavy metals by encapsulating them within their cell membranes.

Hazardous metals can be eliminated or converted into harmless molecules through the process of bioremediation. This paper provides a comprehensive analysis of the function of microbial remediation in the management of industrial wastewater.

Keywords: *lead, toxic Heavy metals, health risk, industrial waste water, microbial remediation.*

INTRODUCTION

On a global scale, significant volumes of wastewater are produced as a result of industrial growth and the trend of urbanization. Approximately 22% of the world's water supply is consumed by the industrial sector. About 80% of all generated wastewater is discharged into rivers, negatively impacting aquatic ecosystems and harming the environment. According to The Trade Council, Embassy of Denmark, India, 2015, India produces approximately 44 million litres of industrial effluent each day, with about 6.2 billion litres released into the nation's natural water bodies without treatment (Dutta et al. 2021). A wide array of industries plays a crucial role in the introduction of metals into wastewater. These include sectors such as chemical production, distilleries, sugar processing, food and dairy, paper and pulp, textile bleaching and dyeing, mining and quarries, battery manufacturing, nuclear power, organic chemicals, leather and tannery, iron and steel, soap and detergent, electric power generation, metal refining, pesticide and biocide production, petroleum and petrochemical, pharmaceuticals, metal processing, and electroplating. Copper (Cu), Zinc (Zn), Cadmium (Cd), Chromium (Cr), Mercury (Hg), Iron (Fe), Lead (Pb), Nickel (Ni), and Arsenic (As) are metals released by these industries (Awuchi et al. 2020). The lack of adequate wastewater treatment facilities, marked by electrical malfunctions, poor maintenance, and a shortage of skilled personnel, leads to the frequent release of untreated wastewater into water bodies, negatively impacting local aquatic ecosystems. Contaminated water supplies, particularly groundwater, represent a significant risk to human health and contribute to ecological degradation (Bora et al. 2019; Dutta et al. 2021). As a result, with an increasing number of enterprises regularly

using resources like water, there is a growing amount of wastewater released into the environment, which includes a range of harmful heavy metals. The management of industrial wastewater has become a significant concern on a global scale. The treatment of wastewater through various methods has been the subject of extensive scientific inquiry and analysis to date (Lee et al. 2019; Alalwan et al. 2020; Babincev et al. 2020; Mustafa et al. 2020). The ongoing application of these techniques reveals considerable challenges, such as low efficiency, the requirement for substantial amounts of solvents and reagents, and the generation of secondary pollutants, including waste residues, sludge, wastewater, and toxic compounds (Gebretsadik et al.2020). As a result, the need for efficient wastewater treatment that is both cost-effective and has minimal negative impact on the environment has become critically important worldwide (Dutta et al. 2021). Traditional approaches, including physical, chemical, and thermal treatments, exhibit several significant drawbacks, such as the production of harmful byproducts, the need to transport contaminated soil or water for cleanup, elevated treatment expenses, and inadequate recovery of natural ecosystems and wildlife. The use of bioremediation techniques that incorporate organisms like microorganisms (including bacteria, fungi, and Actinomycetes) or their byproducts can effectively reduce and neutralize pollutants in industrial wastewater through conventional biological processes, whether aerobic or anaerobic (Arora 2018; Saxena et al. 2016).

RESULT AND DISCUSSION

Almost every nation around the world has lead, with approximately 800 locations showing significant levels of lead exposure. The World Health Organization reported in 2018 that high levels of lead contamination have put approximately 26 million people at risk and led to 540,000 deaths annually, with developing countries bearing a disproportionate share of this crisis (Rahman et al. 2019). The main sources of heavy metals, especially lead, in wastewater include the chemical, distillery, food and dairy, sugar, pulp and paper, petroleum, soap, detergents, and leather/tannery industries, stemming from various industrial processes and operations (Dutta et

al. 2021). Lead (Pb) ranks as the second most harmful heavy metal found on Earth in terms of pollution (Pratush et al. 2018). Lead levels exceeding the critical threshold have a significant impact on all morphological, physiological, and biochemical systems (Kushwaha et al. 2018). The evaluation of lead toxicity at various trophic levels within the food chain is presently conducted through a range of quantitative metrics. Lead can build up in the soil or move to the aerial parts of plants if it seeps into the soil from any source and subsequently enters the plant's root system (Kumar et al. 2020). Soil pollution negatively impacts plants by disrupting nutrient uptake, changing water dynamics, and producing reactive oxygen species (ROS), which obstruct photosynthesis and cause cell death, resulting in a significant reduction in crop yield (Zulfiqar et al. 2019). Evidence suggests that lead interferes with enzymes related to germination, such as amylase and protease, negatively impacting the development of radicals and hypocotyls. Furthermore, the decreased mobilization of stored nutrients results in lower radical synthesis, compromised proteolytic functions, and disturbances in cellular osmoregulation, all of which obstruct germination and seedling development in the presence of lead. Lead contamination negatively affects the structure and function of seeds, hindering the initial growth and germination of crops. Lead primarily enters the human body through ingestion and inhalation. Regardless of how it enters the body, lead poses a threat to almost every organ. Acute issues stemming from lead poisoning occur far less frequently compared to long-term conditions. Children show similar effects at lower levels of lead exposure, even though the harmful implications are the same for both adults and children (Rahman et al. 2019; Gidlow 2015; Kumar et al. 2020). Both the peripheral and central nervous systems exhibit susceptibility to the impacts of lead (Flora et al. 2012). Exposure to lead, whether in acute or chronic forms, leads to haemolytic anaemia, hypertension, and cardiovascular disease (Gidlow 2015). Acute lead exposure in the renal system leads to the development of Fanconi syndrome, whereas chronic exposure causes nephropathy. Extended exposure to lead poisoning negatively impacts the reproductive systems of both males and females, resulting in

reduced libido, abnormal sperm production, and infertility in men, while contributing to miscarriage and premature delivery in women (Rahman et al. 2019; Flora et al. 2011). The increasing occurrence of environmental degradation, the anticipated number of polluted sites, and the ongoing efforts to identify them have led to intensified global initiatives aimed at remediating many of these areas in recent years. This is carried out either to reduce any negative health or environmental effects caused by pollution or to prepare the area for future reconstruction or rehabilitation (Luka et al. 2018; Arora 2018). Bioremediation represents a sustainable approach that, due to its cost-effectiveness and environmental advantages, can effectively address environmental degradation. Bioremediation is a technology that employs biological processes to remove or reduce contaminants in the air, soil, and water. The method utilizes an organism obtained from an external system or the relevant environment to reduce or eliminate the hazardous component. (Sher et al., 2019; Luka et al., 2018; Arora, 2018) (Ashraf et al., 2018; Saravanan, 2022). Microorganisms that employ enzymes to break down pollutants into harmless molecules play a vital role in most bioremediation processes. Bioremediation often necessitates adjustments to environmental conditions to promote microbial growth and degradation, as it can only take place in settings that support microbial activity (Karigar et al. 2011). It is essential that isolated microorganisms can be grown in almost any environmental condition. Microbes exhibit remarkable adaptability, thriving in environments characterized by extreme cold, intense heat, arid deserts, aquatic settings, high oxygen levels, anaerobic conditions, exposure to harmful substances, and diverse waste streams. The two fundamental elements consist of an energy source and a carbon supply (Luka et al. 2018). This method relies on various environmental factors and inputs, including nutrients, oxygen, and other modifications, to enhance microbial activity for the purpose of lead remediation (Gong et al. 2018). Various microbial strains utilize immobilization methods for the bioremediation of lead. The bacteria have evolved various defense mechanisms that enable them to withstand the toxic effects of lead (Pratush et al. 2018).

Microbial remediation entails the use of native or foreign microorganisms to reduce lead concentrations in the environment. Alcaligenes sp., Bacillus firmus, Bacillus licheniformis, Enterobacter cloacae, Escherichia coli, Micrococcus luteus, Pseudomonas fluorescens, Aspergillus niger, Aspergillus terrus, Aspergillus versicolour, Neurospora crassa, Penicillium canescens, Penicillium chrysogenum, Penicillium decumbens, Penicillium simplicissimum, Saccharomyces cerevisiae, and Salmonella typhi are notable species that demonstrate the ability to biotransform and bind lead from contaminated environments (Kumar et al. 2020; Pratush et al. 2018). The Bacillus subtilis mutant strain B38 shows considerable potential for the extraction of heavy metals, especially lead, in China (Wang et al. 2014). The Aspergillus niger strain SY1 demonstrated a remarkable capability to remove 99.5% of lead from contaminated sediment through the process of bioleaching (Zeng et al. 2015). Notable organisms involved in biotransformation of lead are Pseudomonas aeruginosa, Synecochoccus sp., and E. coli, which possess the capability to remediate lead via biosorption. In Pseudomonas aeruginosa, Pb2+ associates with molecules featuring carbonyl, phosphate, hydroxyl, and amino groups within the cell wall, whereas in Synecochoccus sp., it interacts with amide, amino, hydroxyl, or carboxyl groups present in the cell wall. About 97% of the Pb2+ in E. coli is contained within the cell membrane. Klebsiella michiganensis, Providencia rettgeri, Raoultella planticola, and Serratia sp. are acknowledged for their capacity to biosorb lead from both mono- and mixed metal solutions through the secretion of extracellular polymeric substances (EPS) (Arashiro et al. 2018). Potential bio-sorbents include the fungal biomass of Lepiotahystrix, Aspergillus niger, Aspergillus terreus, and Trichoderma longibrachiatum (Kumar et al. 2020).

CONCLUSION

An in-depth examination reveals that the substantial discharge of lead from various sectors via wastewater significantly affects the entire ecosystem. The adverse impacts of industries have underscored the urgent need for sustainable technology solutions. The physio-chemical therapy techniques incorporate

numerous chemicals while maintaining an environmentally friendly approach. To effectively address and remove the solid wastes and wastewater generated by industries containing heavy metals, bioremediation technologies could serve as a viable solution. These technologies are cost-effective, environmentally friendly, and present a promising approach to improving environmental quality. This study highlights several microorganisms that have shown the capability to eliminate or remediate harmful issues found in waste materials produced by industrial processes. In light of the vast array of microorganisms available and the numerous eco-friendly and cost-effective methods at our disposal, it is concerning that industrial wastes and wastewater still frequently contribute to environmental pollution and toxicity challenges. On that note, this study serves as a means to enhance the community's goodwill. It is essential to implement appropriate measures to manage heavy metals, especially lead, in an eco-conscious manner. Reducing the reliance on hazardous chemicals is crucial, and it is important to raise awareness among government entities and other organizations regarding this critical matter.

CONFLICT OF INTEREST

There is no conflict of interest related to the study.

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The Potential Therapeutic Applications of Copper and Its Complexes and Copper Chelation Therapy

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ABSTRACT

Copper, a vital trace element, is integral to various biological functions, including cellular respiration, free radical detoxification, iron metabolism, neurotransmitter production, and angiogenesis. This transition metal exhibits distinctive redox characteristics that render it a versatile therapeutic agent across various disease domains. This review study examines the potential functions of copper and its complexes in anti-cancer, anti-microbial, and anti-inflammatory therapy, as well as the application of copper nanoparticles in wound healing processes. The concentration of copper must be meticulously monitored, as any deviation in its homeostasis can lead to abnormalities. One of the most promising strategies for sustaining physiological copper levels is copper chelation therapy, which has emerged as a vital treatment for neurodegenerative disorders, including Wilson's disease, Alzheimer's disease, as well as conditions such as diabetes mellitus and idiopathic pulmonary fibrosis. Besides demonstrating significant biocidal qualities, copper is also linked to skin health. The comprehensive understanding of the bioinorganic

characteristics of copper and its complexes, alongside the novel prospects presented by advancements in medicinal chemistry, is facilitating the identification of a new category of highly efficacious pharmaceuticals with minimal side effects, potentially enhancing clinical research and practice significantly.

Keywords: *Copper, copper complexes, anti-cancer agent, anti-microbial agent, copper nanoparticles, neurodegenerative disorders, anti-arthritic drugs, copper chelation therapy.*

INTRODUCTION

Copper, similar to essential amino acids and essential fatty acids, is a vital microelement required for normal metabolic processes (Brewer,2009). Nonetheless, it cannot be produced via de novo pathways, making dietary consumption essential. Copper demonstrates considerable metabolic activity, serving as either a vital trace element or a key component of various substances introduced externally to individuals. Initially, it attaches to proteins such as albumin and ceruloplasmin; thereafter, it interacts with different ligands to create complexes that connect with biomolecules, primarily proteins and nucleic acids (Delimaris et al.,2008). Copper-binding proteins, including cytochrome oxidase, copper-zinc-superoxide dismutase, lysyl oxidase, tyrosinase, and dopamine-beta-monooxygenase, play a crucial role in vital biological processes such as mitochondrial respiration, antioxidant defense, extracellular matrix cross-linking, pigmentation, and neurotransmitter biosynthesis (Kim et al.,2008). Copper is primarily found in organs that exhibit high metabolic activity, such as the liver, kidneys, heart, and brain. About five percent of the total copper in the body is found in serum, with nearly 95% of this quantity associated with ceruloplasmin. The standard range of copper levels in healthy individuals is roughly 50–120 mg. The regulation of copper concentration is essential, as unbound copper acts as a strong oxidant, resulting in the formation of highly reactive hydroxyl radicals that can harm proteins, lipids, and DNA (Uriu-Adams & Keen,2015). Homeostatic processes, such as absorption, elimination, and bioavailability, meticulously regulate the concentration of copper in cells (Kaplan &

Maryon,2016). The primary protein responsible for copper import is Copper transporter 1, which is situated on the cell membrane. ATPase copper-transporting alpha (ATP7A) and ATPase copper-transporting beta (ATP7B) are essential for maintaining copper balance, enabling the movement of copper to the secretory pathway and the removal of excess copper from the cell (Bandmann et al.,2015). Copper readily transitions between its two oxidation states, Cu(I) and Cu(II). Copper ions in the body demonstrate notable redox activity and promote electron transfer in both the +1 and +2 oxidation states, essential for enzyme-catalyzed processes, including biological redox reactions. Copper serves dual roles as both a valuable and harmful component within a cell. A developing collection of in vitro and in vivo studies indicates that copper-based mechanisms could act as promising therapeutic targets for a range of diseases. The current investigation primarily focuses on the regulation of copper levels within biological systems. The connection between copper and tumors reveals that individuals with cancer show increased copper concentrations in their serum and tissues, implying that the presence of malignancy might interfere with the body's regulation of copper levels. Studies indicate that copper facilitates angiogenesis, metastasis, and the development of tumors (Harvey et al.,2013; Tapiero et al.,2003). Multiple investigations suggest that increased levels of copper can have detrimental impacts on cancer cells. Oral copper chelators have demonstrated the ability to inhibit tumor growth and metastasis in both human patients and animal cancer models (Lopez at al.,2019). Copper-based compounds demonstrate promising anti-cancer properties. Copper is acknowledged as a vital component of the innate immune system, functioning as a key line of defense against invading pathogens. Small-molecule ligands offer a promising avenue for therapeutic mimicry of the immune system; however, the use of free copper is not appropriate due to its absence of host-pathogen selectivity. Moreover, numerous studies have highlighted the antiviral and antibacterial characteristics of Cu (II) complexes (Borkow & Gabbay,2014). Extended contact with copper surfaces has demonstrated a decrease in the infectivity of the influenza A virus (Noyce et al.,2007). Numerous well-known

therapeutic agents, including pyrithione and disulfiram, exhibit notable inhibitory effects that are dependent on copper. The implementation of high-throughput screens resulted in the identification of multiple novel compounds that function as copper-dependent inhibitors targeting Staphylococcus aureus and Mycobacterium tuberculosis (Jimenez-Garrido,2005). Copper complexes show promise as anti-inflammatory agents and could be important in anti-arthritic therapies, as indicated by multiple reviews. Cupric carbonate and cupric complexes show effectiveness in reducing inflammation. A variety of Cu (II) complexes formed with non-steroidal anti-inflammatory drugs (NSAIDs) exhibit improved anti-inflammatory and antiulcerogenic effects, as well as decreased gastrointestinal toxicity, when compared to traditional uncomplexed medications (Crisponi et al.,2010). Copper chelation therapy serves as a viable treatment approach for conditions in which an imbalance of copper plays a crucial role in disease development. This encompasses idiopathic pulmonary fibrosis, diabetes, neurodegenerative conditions such as Parkinson's and Alzheimer's, as well as genetic disorders related to copper metabolism, including Wilson's disease. Copper chelating agents, such as trientine are considered safe for application in individuals with Wilson's disease and those undergoing cancer treatment (Weiss et al.,2013). Copper serves as a bioactive nanoparticle, demonstrating intricate functions across diverse cellular environments. This plays a crucial role in the mechanisms of action of growth factors and cytokines, being fundamentally involved in every stage of the wound healing process. Copper plays a crucial role in angiogenesis, skin regeneration, and enhancing the healing process (D et al.,2021). Although there is a wealth of information on the effects of copper on individuals, the function of copper complexes in the medical field is still not fully comprehended, and the clinical significance of copper status is not consistently well-defined. These substances seem to hold significant importance in medical processes, and their value may have been overlooked. This study seeks to examine the current body of literature regarding the physiological roles of copper, with an emphasis on the biochemical effects of copper complexes and their possible therapeutic uses.

METABOLISM AND REGULATION OF COPPER

The Copper Transporter 1 protein (CTR1) functions as the main pathway for the uptake of copper into cells (Galler et al.,2020). Copper is transported to CCS, COX17, and ATOX1 after it enters the cell. SOD1 (superoxide dismutase 1) obtains copper from CCS, playing a crucial role in safeguarding against oxygen toxicity. Cytochrome c oxidase (CCO), crucial for mitochondrial respiration, obtains copper from COX17 (Kim et al.,2008). The transport of copper into the cell nucleus occurs via ATOX1, which subsequently promotes the expression of G1/S-specific cyclin D1, thereby aiding in the process of cell division (Itoh et al.,2008).

The Golgi network's complex transport mechanisms enable ATOX1 to regulate the subcellular distribution of copper, facilitating its transfer to ATP7A and ATP7B throughout the network (Gudekar et al.,2020). To prevent copper toxicity, MT and GSH sequester surplus copper within the cell. Additionally, ATP7A (ATPase copper transporting alpha) and ATP7B (ATPase copper transporting beta) primarily serve to enable the efflux of copper ions from cells. Copper plays an essential role as a transition metal, participating in numerous critical processes; however, it can exhibit toxic effects in biological systems if not properly regulated. While Cu (II) represents the predominant redox state in the bloodstream, Cu(I) is the reduced form that is more commonly observed within cells. Cu (II) undergoes reduction to Cu(I) through various mechanisms prior to absorption by the cells. To avert cellular damage caused by copper, biological systems employ specialized proteins that meticulously regulate the absorption, transport, and excretion of copper, as well as its allocation to specific copper-binding protein sites where this metal is essential (Macomber & Imlay,2009).

COPPER AS ANTI-CANCER AGENT

Patients with acute leukaemia exhibit elevated levels of copper in their serum or plasma (Tisato et al.,2010). An increase in bone marrow blast cells was associated with a rise in serum copper levels (Deng et al.,2006). The relationship between lowered serum copper levels and

the alleviation of symptoms or remission after treatment has streamlined the process of making predictions based on blood copper assessments. The following image demonstrates the relationship between copper and cancer. A key characteristic of cancer cells is their ability to evade cell death, which plays a significant role in their resistance to treatment. Inducing cell death through the targeting of copper ions can facilitate the differentiation of cancer cells from healthy cells, given the increased copper enrichment observed in cancerous tissues. Currently, a highly discussed area in the advancement of anti-cancer therapies is the focus on copper targeting. Currently, there are two main strategies for addressing cancer that focus on copper targeting. Initially, we have copper chelators such as trientine, TTM, and D-penicillamine (Nagai et al.,2012; Tawari et al.2015). These medications function to combat cancer by interacting with copper and reducing its bioavailability. Copper ionophores encompass compounds such as disulfiram and elesclomol (Tsvetkov et al.,2022). The generation of reactive oxygen species, inhibition of the proteasome, and induction of apoptosis enable these carriers to elevate intracellular copper ion levels, thereby exhibiting anti-tumour effects (Liu et al.,2015).

COPPER AS ANTI-MICROBIAL AGENT

Copper possesses inherent anti-microbial characteristics that effectively combat bacteria including Escherichia coli and Staphylococcus aureus, as well as viruses such as SARS-CoV2, Influenza A virus, and norovirus, along with fungi like Aspergillus and Candida species. Copper nanoparticles exhibit anti-microbial properties (Kaweeteerawat et al.,2017). The activity of copper nanoparticles is influenced more by their size than by their concentration; smaller nanoparticles demonstrate greater effectiveness against microbes. The generation of reactive oxygen species, which harm membranes, serves as the main mechanism behind bactericidal action (Pham et al.,2013; Yang et al.,2014). The primary mechanism through which the copper surface exerted its antiviral effects was ion release, leading to RNA degradation and membrane breakage in enclosed viruses (Warnes et al.,2015). The primary

processes involved in copper infiltration in fungi are believed to include the absorption of copper ions and the physical breakdown of the membrane (Muñoz-Escobar et al.,2020).

ROLE OF COPPER IN ANTI-INFLAMMATORY THERAPIES

Copper complexes constitute a unique class of anti-arthritic agents for two main reasons. Studies involving animals have shown that these agents are effective anti-ulcer treatments when evaluated against currently utilized ulcerogenic medications. Secondly, when compared to long-term treatment with current medications, short-term Cu complex therapy led to lasting remissions in the treatment of human rheumatic diseases (Soylak & Kirnap,2010). The dual aspects of copper complexes' biological activity, coupled with their proven anti-inflammatory properties in various animal models, indicate that therapy involving copper complexes could provide benefits compared to existing medication strategies. Cupric carbonate and cupric complexes of acetic, lauric, oleic, caprylic, butyric, sebacic, lipoic, and cinnamic acids showed effectiveness in animal models of inflammation (Patel et al.,2010).

COPPER CHELATION THERAPY

A chelator is a chemical entity that selectively interacts with particular atoms or ions, resulting in the formation of a stable, cyclic complex structure. Metal chelating compounds act as growth enhancers in aquaculture and are utilized as additives in various products such as cleaning agents, cosmetics, plastics, fertilizers, and nutritional supplements. Chelation therapy is utilized to eliminate toxic metals from the body as well as from soil environments. Clinically significant copper deficiency and copper overload toxicity are uncommon and mainly linked to genetic disorders in copper transport (Ding et al.,2011). Furthermore, there is a link between diabetes and neurological conditions with copper dyshomeostasis, resulting in its incorrect distribution. Different copper chelating agents, including D-penicillamine (S)-2-amino-3-mercapto-3-methylbutanoic acid, tetrathiomolybdate, and trientine, influence cellular copper levels via unique

mechanisms (Lu,2010). The primary therapeutic approach of copper chelation therapy emerged after thorough investigation into the copper imbalance associated with Wilson's disease. This has greatly decreased morbidity and made Wilson's disease manageable (Mohr,2019). The focus is on assessing novel chelating agents and formulations to improve blood-brain barrier permeability, minimize adverse effects, and enhance patient compliance (Maher et al.,2015). Neurological disorders, including Alzheimer's disease, are characterized by the extracellular accumulation of β-amyloid protein and its buildup in the brain (Sensi et al.,2018). Studies suggest that metal chelating agents may decrease excess β-amyloid protein, indicating that copper chelating agents like D-penicillamine could play a potential therapeutic role in individuals with Alzheimer's disease (Squitti et al.,2002). Studies on idiopathic pulmonary fibrosis show that administering tetrathiomolybdate leads to lower serum ceruloplasmin levels, which is associated with a decrease in lung fibrosis (Ovet et al.,2014). Individuals diagnosed with diabetes mellitus demonstrate higher copper concentrations when contrasted with those who are healthy. The utilization of copper chelators to sustain copper homeostasis could represent a potential therapeutic approach for diabetes management. Metformin, acknowledged as a primary treatment for type II diabetes, diminishes the likelihood of vascular complications linked to the condition and shows promise as a therapeutic agent with chelating capabilities. Metformin demonstrates a greater affinity for copper and interacts with several transitional metals (Repiš˘cák et al.,2014; Foretz et al.,2019). In a mouse model of type II diabetes, TM treatment leads to a notable decrease in insulin resistance (Tanaka et al.,2009).

APPLICATION OF COPPER TO IMPROVE THE WELL-BEING OF SKIN

Copper is essential for skin regeneration and angiogenesis, primarily through the induction of vascular endothelial growth factor (VEGF) and the action of hypoxia-induced factor-1-alpha (HIF-1α) (Zhou et al.,2020; Wu et al.,2019). This occurs through the elevation of HIF-1α expression and its

subsequent binding to essential motifs within the promoter and potential enhancer regions of genes regulated by HIF-1. The antibacterial properties and low toxicity profile of copper nanoparticles make them excellent candidates for incorporation into wound dressings. Copper is crucial in the healing process under controlled conditions, as it enhances the expression of extracellular matrix components such as fibrinogen, collagen synthesis, and integrins, which serve as the main facilitators of cell attachment to the extracellular matrix (Sen et al.,2002).

CONCLUSION

Copper and its complexes have a wide range of potential applications in medicine, including wound healing, microbiology, cancer treatment, and anti-inflammatory therapies. Copper exhibits remarkable versatility due to its distinctive redox properties and capacity to engage with biomolecules; however, careful regulation of its concentration is essential to prevent toxicity. Studies show that copper acts as an effective therapeutic agent for various disorders and plays a vital role in maintaining physiological systems. Copper chelation therapy presents intriguing strategies for addressing conditions like idiopathic pulmonary fibrosis, diabetes, and neurological disorders. Copper nanoparticles exhibit significant promise as antibacterial agents and in facilitating skin regeneration, thus expanding their therapeutic uses. Although progress has been made, additional investigation is necessary to comprehensively grasp the therapeutic potential of copper, given the complexities surrounding its clinical application in medicine. Progress in bioinorganic chemistry and clinical studies is anticipated to drive the creation of novel copper-based therapies, potentially leading to fewer side effects and enhanced effectiveness.

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Anticancer Properties Of Selenium: Mechanisms, Applications, And Future Perspectives

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ABSTRACT

Selenium, a crucial trace element, has garnered attention for its potential to inhibit cancer. Its various biological effects, including redox regulation, immunological modulation, and selective cytotoxicity, render it a cancer preventive and therapeutic agent. Selenium compounds such as selenomethionine, selenite, and selenium nanoparticles have demonstrated the ability to induce apoptosis, suppress cell proliferation, and modify oxidative stress pathways in breast, prostate, and lung malignancies. Evidence indicates that selenium functions synergistically with selenoproteins, as these molecules support cellular homeostasis and inhibit tumor development, hence augmenting selenium's anticancer efficacy. Toxicity in a dose-dependent manner, inconsistent bioavailability, and varying clinical outcomes provide challenges that necessitate further exploration. This study elucidates the underlying mechanisms of action for the anticancer effects of Selenium, its clinical indications, and future prospects, particularly focusing on Selenium-based nanotherapeutics and their application in personalized medicine.

Keywords**:** *Selenium, Anticancer Properties, Chemoprevention, Redox Regulation, Selenoproteins, Apoptosis*

INTRODUCTION: Due to the challenges in monitoring and treating the disease, cancer remains one of the leading causes of mortality worldwide. Moreover, with surgical intervention, chemotherapy, radiation, and receptor modification therapy are commonly employed modalities for cancer treatment, despite their potential for significant adverse effects. The motivation generated by such constraints will be the pursuit of novel medicinal agents, particularly those derived from natural sources or dietary components, which are regrettably less hazardous. Selenium, a significant non-metal, has emerged as a possibility for cancer prevention and treatment among other substances. The incorporation of selenoproteins into specific selenoproteins, vital for antioxidant defense and immunological modulation, facilitates the maintenance of cellular homeostasis and redox buffering, with selenium serving this function. Population and cross-sectional studies have demonstrated a substantial connection between selenium concentration and cancer incidence rates, indicating its potential utility as an anticancer agent.

Selenium combats malignancies by mediating many activities, including oxidative stress, inducing apoptosis, and inhibiting cell proliferation. It possesses the distinctive capability of operating on a substantial tumor while preserving the adjacent healthy cells. Selenomethionine, sodium selenite, and selenium nanoparticles have demonstrated efficacy in both clinical and preclinical investigations and may be utilized in conjunction with several other cancer therapies. This review aims to elucidate the anticancer properties of selenium, focusing on its mode of action, applications in oncology, and inherent limits. It also examines selenium-based methodologies in personalized medicine and proposes potential applications for cancer treatment in the future.

Roles of Selenium in Cancer Prevention and Therapy

Role	Mechanism/Function	Examples/ Implications
Oxidative Stress Regulation	Neutralizes reactive oxygen species (ROS)	Prevents DNA damage and reduces tumor progression.

Redox Balance Maintenance	Incorporation into selenoproteins (e.g., GPxs, TrxRs)	Protects cells from oxidative damage and maintains cellular homeostasis.
Apoptosis Induction	Triggers programmed cell death in cancer cells	Selective targeting of tumor cells without harming normal cells.
Cell Proliferation Inhibition	Disrupts cell cycle and inhibits tumor growth	Effective against various cancers, including breast, prostate, and lung.
Immune Modulation	Enhances immune system activity	Promotes immune surveillance and destruction of cancer cells.
Anti-inflammatory Effects	Reduces inflammation-associated tumorigenesis	Limits tumor microenvironment conducive to cancer growth.
Gene Regulation	Influences DNA repair and gene expression	Reduces genetic instability and supports therapeutic outcomes.

Table. 1: Roles of Selenium in Cancer Prevention and Therapy

CHEMISTRY AND BIOLOGICAL ROLES OF SELENIUM:

Selenium is essential for biological processes and exists in various chemical forms. The chemical considerations may encompass organic forms like selenomethionine and selenocysteine, in addition to inorganic forms including selenium nanoparticles and sodium selenite. These forms enhance bioavailability and biological activity across different physiological processes, demonstrating significant reactivity. The incorporation of this element into selenoproteins, including glutathione peroxidases (GPxs), thioredoxin reductases (TrxRs), and iodothyronine deiodinases, plays a crucial role in maintaining redox

homeostasis, supporting oxidative defense mechanisms, and regulating hormone metabolism within the body. Selenium encompasses a wide array of biological properties, including its roles in antioxidant activity and immune system modulation. This element of selenoproteins targets reactive oxygen species to maintain a stable redox state and aid in the repair of cells harmed by oxidative conditions and inflammation. In conjunction with these processes, Selenium modulates gene expression and the cell cycle, highlighting its significance in cellular stability. The significance of these properties is especially pronounced in the realm of cancer, where oxidative stress and redox dysregulation are pivotal factors in tumor progression. Selenium demonstrates its role as a preventive or mitigating agent by modifying these processes [Radomska et al. 2021].

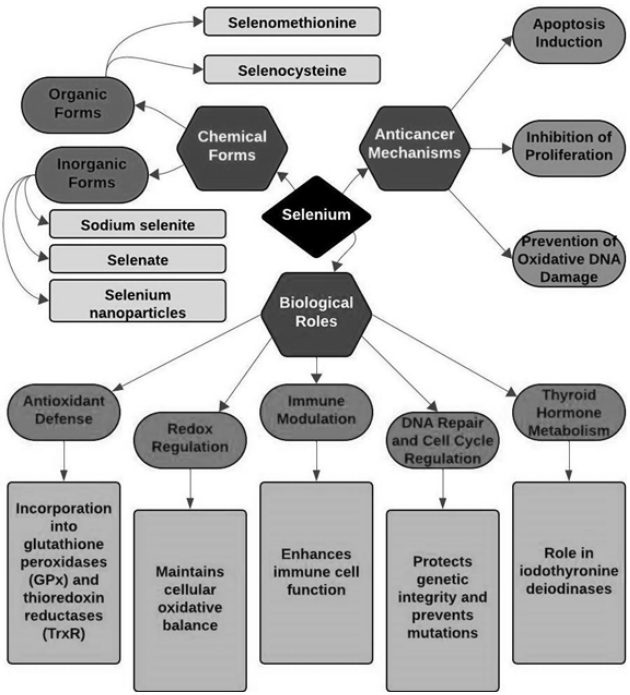

Figure 1. Chemistry and Biological Roles of Selenium

MECHANISMS OF ANTICANCER ACTIVITY:

Selenium demonstrates its anticancer properties via a variety of mechanisms, influencing several pathways that play a role in tumor development and progression.

Oxidative Stress Regulation: Selenium serves as an essential element of selenoproteins, including glutathione peroxidases (GPx) and thioredoxin reductases (TrxR), which are crucial in counteracting reactive oxygen species (ROS). Through the reduction of oxidative damage to DNA and cellular components, Selenium plays a crucial role in minimizing mutagenic events and inhibiting tumorigenesis. Furthermore, the selective cytotoxicity exhibited towards cancer cells is accomplished by restoring redox homeostasis. Selenium compounds demonstrate significant efficacy in inducing apoptosis within cancer cells. These compounds engage both intrinsic (mitochondrial) and extrinsic (death receptor-mediated) apoptotic pathways. This entails the modulation of apoptotic regulators, including the upregulation of pro-apoptotic proteins (e.g., Bax) and the downregulation of anti-apoptotic proteins (e.g., Bcl-2), resulting in programmed cell death. Selenium interferes with the growth of cancer cells by causing cell cycle arrest at critical checkpoints, including the G1/S and G2/M phases. The regulation of cyclin-dependent kinases (CDKs) and their inhibitors mediates this effect, which in turn prevents the replication of damaged DNA and restricts tumor growth.

DNA Repair and Genomic Stability: Selenium plays a crucial role in enhancing genomic stability by mitigating oxidative stress and supporting the function of DNA repair enzymes. This mechanism inhibits the buildup of mutations and chromosomal abnormalities, which are characteristic features of cancer advancement. Selenium demonstrates anti-angiogenic effects by reducing the levels of pro-angiogenic factors, including vascular endothelial growth factor (VEGF), thereby inhibiting tumor angiogenesis. The interruption in the formation of blood vessels restricts tumors from receiving vital nutrients and oxygen, consequently hindering their growth and spread.

Immunomodulation: Selenium boosts immune surveillance by elevating the activity of natural killer (NK) cells and T lymphocytes. This enhances the immune system's capacity to recognize and eradicate cancer cells. Additionally, Selenium's ability to combat inflammation contributes to the reduction of tumor-promoting inflammatory responses within the tumor microenvironment. Selenium plays a role in the expression of cancer-related genes by utilizing epigenetic mechanisms, including DNA methylation and histone modification. This approach reinstates the activity of dormant tumor-suppressor genes and inhibits oncogenes, effectively stopping the advancement of cancer. The interaction with traditional treatments: Selenium has shown potential in improving the effectiveness of chemotherapy and radiotherapy by making cancer cells more responsive to these treatments while safeguarding normal tissues from damage caused by therapy.

Selenium Nanoparticles (SeNPs): Recent findings indicate that Selenium nanoparticles may serve as promising targeted agents in cancer treatment. These nanoparticles exhibit enhanced bioavailability and reduced toxicity in comparison to traditional selenium compounds. They promote the highest levels of ROS production, leading to apoptosis and anti-proliferative effects in cancer cells.

The mechanisms highlighted here emphasize Selenium's promise as both a chemopreventive and therapeutic agent in the field of oncology. The capacity to selectively target cancer cells while maintaining normal cellular function positions it as a compelling subject for further exploration and potential clinical use [Kuršvietienė et al. 2020].

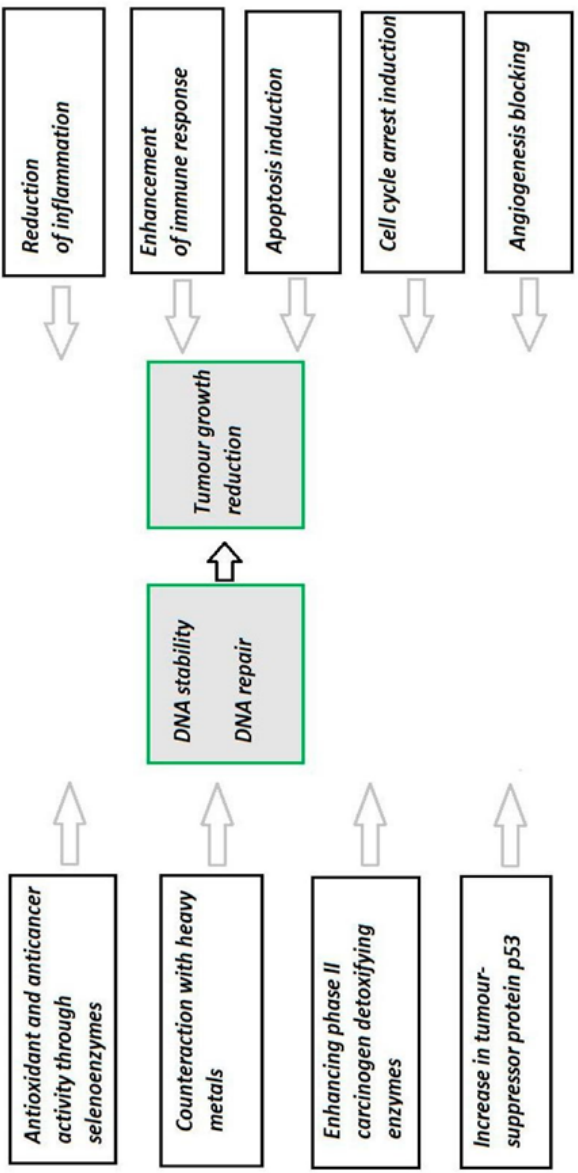

Figure 2. Anticancer effect of selenium may be asserted through various events and pathways in the cell. Data from studies [Wrobel et al. 2016, Chen et al. 2013].

SELENIUM COMPOUNDS IN CANCER TREATMENT:

Sodium selenite is frequently examined for its potential anticancer properties. The findings indicate a promising ability to induce apoptosis in cancer cells, inhibit tumor growth, and enhance the sensitivity of cancer cells to chemotherapy. Research indicates that it could improve the efficacy of standard therapies such as radiation and chemotherapy. Seleno-methylselenocysteine (SeMSC), a naturally occurring selenium compound, has been investigated for its potential to inhibit the growth of various cancer cell types, including those associated with prostate, colon, and breast cancers. The mechanism is thought to involve the modulation of antioxidant pathways, inhibition of DNA damage, and regulation of tumor suppressor genes. Methylseleninic Acid (MSA) represents a selenium-based compound that exhibits noteworthy anticancer properties. Evidence indicates that it can trigger cell cycle arrest, apoptosis, and autophagy in cancer cells. It has shown synergistic effects when used alongside other chemotherapeutic agents, boosting their anticancer activity [Gandin et al. 2018].

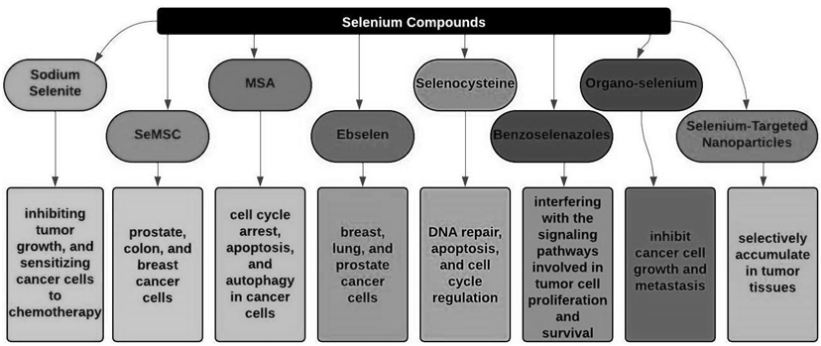

Figure 3. Selenium Compounds in Cancer Treatment

Ebselen is a synthetic organoselenium compound known for its anti-inflammatory, antioxidant, and anticancer properties. This has been examined for its potential to suppress tumor growth and trigger apoptosis in various

cancer cell lines, such as those from breast, lung, and prostate cancers. Selenocysteine (Sec), the amino acid variant of selenium, has been investigated for its possible applications in cancer treatment. This substance exhibits properties that combat oxidative stress and cancer, playing a crucial role in numerous cellular mechanisms, including the repair of DNA, programmed cell death, and the regulation of the cell cycle. Certain investigations indicate that it could be effective in focusing on cancer cells while preserving healthy cells. Benzoselenazoles represent a category of selenium-containing compounds that exhibit notable anticancer properties. These compounds are thought to disrupt the signaling pathways that play a role in the proliferation and survival of tumor cells. Organo-selenium compounds Compounds such as Selenium-containing Phenols and Thiophenols have been studied for their potential to inhibit the growth and spread of cancer cells. These compounds frequently function by triggering oxidative stress or influencing critical molecular targets that play a role in cancer advancement. Nanoparticles that incorporate selenium have surfaced as an innovative strategy for the targeted delivery of drugs in the treatment of cancer. The design of these selenium-based nanoparticles allows for selective accumulation in tumor tissues, which enhances the bioavailability of selenium and minimizes systemic toxicity [Shelar et al. 2021].

LIMITATIONS AND CHALLENGES:

Selenium Toxicity and Dose-Dependent Effects: Selenium compounds exhibit dose-dependent toxicity, with elevated dosages potentially resulting in selenosis, a manifestation of selenium poisoning characterized by symptoms including nausea, vomiting, alopecia, and neurological impairment. The distinction between therapeutic and hazardous levels is crucial, and precise dosing is essential to optimize efficacy while preventing unwanted effects. Prolonged consumption of selenium at elevated levels can result in toxicity, hence restricting its extended application as a therapeutic agent. This is a problem in formulating effective treatment protocols that reconcile anticancer advantages with potential hazards. At elevated quantities, selenium compounds are poisonous

and adversely affect organs including the kidneys, liver, and skin. A significant problem has been determining the correct dosage for clinical use without inducing toxicity.

Variability in Outcomes Due to Selenium Form, Dose, and Cancer Type:

The anticancer efficacy of selenium is contingent upon its chemical form. Organic selenium compounds, such Selenomethylselenocysteine and Methylseleninic acid, may exhibit greater bioavailability and distinct effects relative to inorganic forms such as sodium selenite. This diversity influences their efficacy across various cancer types. The ideal selenium dosage for anticancer efficacy differs among individuals and is contingent upon the specific cancer type. The response of different cancer types to selenium may vary, and the required therapeutic dose for efficacy differs significantly, complicating the establishment of an optimal therapy plan. Various cancer types may exhibit differing levels of reactivity to selenium compounds. Selenium may exhibit a superior inhibitory impact on prostate cancer while demonstrating reduced efficacy in other cancer types, such as breast or lung cancer. This diversity hinders the advancement of broad-spectrum selenium-based cancer treatments. Variations in genetics, metabolism, and personal health circumstances may influence a patient's response to selenium-based therapies. This complicates the prediction of selenium therapy outcomes in various people, even with identical cancer types.

Gaps in Clinical Data and Translational Challenges:

Notwithstanding encouraging preclinical results, the clinical evidence endorsing the application of selenium compounds in cancer therapy remains insufficient. There is an absence of extensive, well conducted clinical trials that conclusively demonstrate the efficacy and safety of selenium-based treatments for cancer. Clinical investigations on selenium have frequently yielded inconclusive results, with certain studies indicating favorable effects, while others demonstrate no significant influence or even negative effects. This contradiction obstructs the broad practical implementation of selenium as a conventional anticancer treatment. Although

considerable knowledge exists regarding the anticancer characteristics of selenium compounds at the cellular level, the exact molecular processes via which selenium operates remain inadequately elucidated. Deficiencies in mechanistic comprehension hinder the optimization of treatment regimens and restrict the capacity to anticipate selenium's behavior in a therapeutic context. Numerous preclinical studies have demonstrated encouraging outcomes with selenium in animal models; however, the application of these results to human cancer treatment has been difficult. The dose, absorption, and toxicity characteristics of selenium might vary considerably among species, complicating the straightforward application of animal studies to human therapy. The regulatory licensing process for selenium compounds in oncological therapy presents an additional barrier. As selenium is classified as a dietary supplement rather than a typical pharmaceutical, the process of obtaining regulatory approval for selenium-based medicines can be intricate and protracted [Garcia-Oliveira et al. 2021].

	LIMITATIONS AND CHALLENGES
Limitation/ Challenge	**Description**
Selenium Toxicity	High doses of selenium can lead to toxicity, including symptoms such as nausea, hair loss, and neurological damage. Toxicity risk increases with prolonged use.
Dose-Dependent Effects	Selenium has a narrow therapeutic window, making it difficult to identify the optimal dose for effective cancer treatment while avoiding adverse effects.
Variability in Selenium Forms	The effectiveness of selenium varies depending on its chemical form (organic vs. inorganic), affecting bioavailability and anticancer efficacy in different cancer types.
Cancer Type Specificity	Selenium compounds may show variable effectiveness across different cancer types, requiring personalized treatment strategies.

Patient-Specific Responses	Individual differences in genetics, metabolism, and overall health influence how patients respond to selenium-based therapies, complicating predictable outcomes.
Limited Clinical Data	There is a lack of large-scale, well-designed clinical trials to confirm the safety and efficacy of selenium compounds in cancer treatment.
Inconsistent Results in Clinical Trials	Clinical trials have yielded mixed results, with some showing benefits and others demonstrating no significant impact or adverse effects, making selenium a less reliable option.
Mechanistic Understanding	The precise molecular mechanisms by which selenium compounds exert anticancer effects are not fully understood, limiting treatment optimization.
Translation from Animal Models to Humans	Findings from animal studies may not be directly applicable to humans due to differences in metabolism, bioavailability, and toxicity profiles.
Regulatory Hurdles	Selenium is classified as a dietary supplement, not a conventional drug, complicating its regulatory approval process for cancer treatment.

Table 2. Limitations and Challenges

FUTURE DIRECTIONS:

The development of selenium nanoparticles presents a promising avenue for enhancing selenium-based therapies. These nanoparticles can be designed to specifically target tumor cells, improving the bioavailability of selenium in cancerous tissues while reducing systemic toxicity. Utilizing selenium nanoparticles for targeted delivery systems has the potential to enhance treatment effectiveness while minimizing side effects by focusing the therapeutic action directly at the tumor location. Current investigations focus on enhancing the dimensions, surface properties, and functional modifications of selenium nanoparticles to improve their efficacy in cancer targeting and

controlled release mechanisms. Progress in personalized medicine may result in selenium-based therapies that are specifically designed to align with the genetic makeup, cancer classification, and unique reactions of each patient. Investigating selenium supplementation may involve pinpointing biomarkers that can indicate the efficacy of selenium therapy in patients. By analyzing the distinct genetic and molecular traits of cancer cells, selenium supplementation can be tailored to enhance its efficacy, leading to better patient outcomes while reducing toxicity. Tailored dosing strategies and the choice between organic and inorganic forms of selenium may soon be integrated into routine clinical practice. The integration of selenium compounds with other innovative cancer therapies, including immunotherapy and gene therapy, presents a promising avenue for exploration. For instance, selenium might improve the immune response by increasing antioxidant levels or enhancing the effectiveness of immune checkpoint inhibitors. Furthermore, selenium may have a complementary effect when used alongside gene therapies designed to rectify genetic mutations or improve the body's capacity to target cancer cells. Investigating the integration of selenium with various therapeutic approaches could lead to more effective and comprehensive strategies for cancer treatment.

CONCLUSION:

Selenium compounds exhibit considerable potential as anticancer treatments; nonetheless, their clinical application encounters numerous hurdles, such as toxicity risks, dosage variability, and the necessity for more comprehensive clinical evidence. Ongoing research into new delivery technologies, such as selenium nanoparticles, and the potential for individualized treatment techniques based on individual genetic profiles present promising options for enhancing selenium's therapeutic efficacy. Furthermore, the incorporation of selenium with other medicines such as immunotherapy and gene therapy may improve therapeutic efficacy and offer a more holistic strategy for

cancer treatment. Ongoing research and development may render selenium-based therapies a significant element of individualized, targeted cancer treatment in the future.

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Sources and Effect of Mercury on Human Health

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ABSTRACT

One of the common environmental pollutants that naturally exists on Earth as a result of volcanic eruptions and soil erosion is mercury. Additionally, a variety of human activities, such as the burning of coal in power plants, the smelting of zinc, the artisanal gold mining industry, etc., emit this environmental contaminant. Mercury can be found in various forms, including elemental, organic, and inorganic, and when consumed or administered, it can have a variety of negative health impacts. Mercury can be found in a variety of places, including soil, fish (such as swordfish and shellfish), and more. The mercury is bioaccumulated by the fish through its consumption of planktons and smaller fish. Because of various anthropogenic activities, there is mercury in the soil, which is then absorbed by the rice that is planted there. People who work in dental clinics, in companies that produce pesticides and insecticides, and in alkaline alkali factories are exposed to mercury. When consumed directly, the element is non-toxic; nevertheless, inhaling its vapor or agitated form makes it hazardous. It damages the brain system, liver, kidneys, and lungs. It is very harmful to children's central and peripheral nervous systems. Fetuses are exposed to mercury through placental blood, and nursing newborns also experience serious side effects since they receive mercury through their mothers' milk. This review provides an overview of mercury's physico-chemical

characteristics. In our environment, it draws attention to the various visible and unseen sources of mercury. It explains mercury's pharmacokinetic and pharmacodynamic effects. It also has hazardous impact on humans at all ages.

Keywords: *Mercury, Bioaccumulated, Environmental pollutants, Health Impacts, Pharmacokinetics.*

1. INTRODUCTION

Mercury, a toxic heavy metal, has caused substantial public health catastrophes in Iraq [Afriza, et.al 2013, Bjørklund, et.al 2017] and Minamata Bay, Japan [N.R., at.el 2013]. The therapeutic implications of reduced mercury exposure continue to be a topic of active discussion. Methyl, ethyl, phenyl, and similar groups represent inorganic mercury, which includes metallic mercury, mercury vapor (Hg0), and mercurous (Hg2++) or mercuric (Hg++) salts. Inorganic mercury includes substances in which mercury is chemically linked to carbon-containing structures. The chemical structure affects the pharmacokinetics, clinical significance, and biological behavior of different mercury forms. The many types of mercury undergo interconversion within a living organism. Inhaled elemental mercury vapor is effectively absorbed through the lungs and mucous membranes and is promptly oxidized into different forms; nonetheless, this process is insufficiently swift to avert significant accumulation of elemental mercury in the brain. Methyl mercury is readily absorbed through the gastrointestinal system and accumulates in diverse tissues; nevertheless, its capacity to traverse the blood-brain barrier is inferior to that of elemental mercury. It progressively demethylates to elemental mercury upon entering the brain [UNEP, Global Mercury Assessment, 2013]. Mercury salts are generally distinguished by low absorption, insolubility, and significant stability. The specific type of mercury, dosage, and exposure rate substantially affect human toxicity. Inhalation of mercury vapor predominantly impacts the brain as the principal organ impacted. Methyl mercury is disseminated throughout the body, while mercuric and mercurous chemicals markedly impair the neurological

system and gastrointestinal function (UNEP, Global Mercury Assessment, 2013). Acute exposure to vital mercury vapor can lead to severe pneumonitis, perhaps resulting in death in extreme instances (UNEP, Global Mercury Assessment, 2013). The concentration of toxins differs among various lozenges. Chronic low-level exposure to elemental or other forms of mercury leads to less pronounced symptoms and clinical manifestations, as elaborated below. There is contention on the methodologies for clinical assessment of mercury exposure, as well as substantial debate concerning the clinical implications of exposure to different types of mercury. This research intends to examine the existing data on these motifs and assess the clinical experience with DMPS for the extraction of mercury from the human body.

2. SOURCES OF MERCURY EXPOSURE

The World Health Organization notes that substantial exposure to mercury generally occurs in occupational environments, through the intake of contaminated fish, or via the release of mercury from dental amalgams (Bjørklund et al. 2018, Zulaikhah et.al 2015). The Earth's crust contains about 0.5 parts per million of mercury, mainly found in its elemental state or as sulfides. Stormy activity and gemstone outgassing result in atmospheric exposures. Coal combustion and mining activities, especially those associated with gold and mercury extraction, represent major contributors to atmospheric mercury levels. Atmospheric mercury, when deposited in aquatic environments, is transformed by bacteria into organic forms such as methyl or ethyl mercury. These organic compounds are then taken up by smaller organisms and subsequently ingested by larger fish. Mercury concentrations can accumulate in the tissues of apex predator fish, including marlin, swordfish, and tuna. Human exposure to mercury mainly occurs via the ingestion of organic mercury compounds, such as methyl, dimethyl, or ethyl mercury, primarily sourced from seafood, or through the inhalation of elemental mercury vapor from dental amalgams or occupational environments. The World Health Organization (Bjørklund. et al. 2018, Zulaikhah et.al 2015) and Berglund et al. (Genchi et.al 2017) report that human

exposure to metallic mercury arises from the outgassing of mercury vapor from blend paddings, potentially attaining levels of 2 to 28 micrograms per hand face per day, with an absorption rate of approximately 80%. Mercury is recognized as an infrequent source of mercury vapor (Kretsinger et.al 2013). A documented case of idiopathic thrombocytopenic purpura has been attributed to the vacuuming of exposed mercury, resulting in significant acute exposure to mercury vapor. The primary natural sources of methyl and dimethyl mercury (organic mercury) are freshwater and saltwater fish.

3. PHARMACOKINETICS OF MERCURY EXPOSURE

3.1. Inorganic Mercury

3.1.1 Elemental mercury, also referred to as metallic mercury (Hg0). In comparison to the 7–10% absorption of ingested metallic mercury and the 1% absorption via skin contact, approximately 80% of metallic mercury vapor released from emulsions is absorbed through inhalation. Mercury vapor demonstrates a significant affinity for sulfhydryl groups upon entering the body, resulting in the formation of bonds with sulfur-containing amino acids within the organism. Mercury vapor is conveyed to the brain either in a dissolved state within serum or attached to red cell membranes (Damin. et.al 2009). Metallic mercury readily crosses the placenta and infiltrates the fetal brain after passing through the blood-brain barrier (Houston. et.al 2007, Counter et.al 2004). Upon entering the bloodstream, metallic mercury swiftly oxidizes to form mercuric mercury [UNEP, Global Mercury Assessment, 2013]. Nonetheless, the rate of oxidation is inadequate to avert considerable absorption by the central nervous system while the substance remains in its metallic state. Organ dysfunction may be associated with metallic mercury, which is present in multiple tissues such as the thyroid, bone, myocardium, muscles, adrenal glands, liver, feathers, skin, sweat glands, pancreas, enterocytes, lungs, salivary glands, testes, and prostate. Mercury preferentially interacts with sulfhydryl groups, affecting T cell activity and the binding sites on T cell surfaces. Mercury is present in bone milk and accumulates in the tissues of both the fetus and placenta. Mercuric mercury is the primary form of metallic mercury excreted. The excretory

half-lives of metallic and mercuric mercury differ considerably depending on the organ of deposition and redox state, spanning from a few days to several months, with specific pools like the central nervous system demonstrating half-lives that can exceed several years. No correlation is observed between the concentrations of metallic mercury in the brain and those in hair [UNEP, Global Mercury Assessment, 2013]. The accurate assessment of body burden presents challenges due to these complications.

3.1.2 Mercury in its ionic form (Hg2++). Hg2Cl2 (calomel), a mercury salt, demonstrates low solubility in water and exhibits minimal absorption in the gastrointestinal tract, although it is believed that some may oxidize into more bioavailable forms. In addition to serving as an intermediary between metallic and mercuric mercury, the persistence of mercurous mercury in the body seems unlikely [UNEP, Global Mercury Assessment, 2013]. The correlation between calomel and acrodynia, or pink illness, suggests the occurrence of absorption.

3.1.3 Mercuric chloride ($HgCl_2$), previously employed as a preservative and in photographic processes, can be ingested accidentally or intentionally. Initially, around 2% of the substance is absorbed; however, prolonged exposure may increase absorption due to its corrosive effects on the intestine. Mercuric mercury has limited ability to cross the blood-brain barrier; nonetheless, it can accumulate in the placenta, fetal tissues, and brain through specific transport mechanisms. In the bloodstream, it interacts with proteins such as glutathione and metallothionein. It generally accumulates in the liver, kidneys, and other organs. Excretion occurs via multiple pathways, including urine, feces, sweat, tears, breast milk, and saliva. The half-life of most oral doses of mercuric mercury is 42 days; however, some may remain in the body for prolonged durations.

3.2. Organic Mercury Compounds

Humans predominantly encounter organic mercury compounds, especially methyl mercury, via fish consumption. Methyl mercury demonstrates stability and is readily absorbed

via the skin, lungs, and intestines. It is present throughout the body, with significant concentrations observed in the brain, liver, kidneys, placenta, and fetus. It interacts with sulfhydryl groups, particularly in cysteine. Over a span of 70 days, the body methodically converts methyl mercury into inorganic mercury, with around 90% excreted via feces. Hair mercury reflects recent blood concentrations, with a minor quantity excreted in breast milk. Although ethyl mercury has a shorter excretory half-life, it demonstrates similar behavior. Dimethyl mercury is easily absorbed through the skin and presents considerable health hazards; even slight exposure can lead to lethal consequences.

4. THE EFFECTS OF MERCURY ON HEALTH

Exposure to mercury can result in considerable negative health outcomes, especially related to chemical compounds like methyl mercury. Methyl mercury can enter the body through ingestion, dermal exposure, or inhalation, and it accumulates in fish. Upon absorption, it interacts with protein sulfhydryl groups, thereby disrupting cellular processes and leading to adverse effects. The condition predominantly affects the nervous system, particularly in fetuses and young children, who are more vulnerable and may exhibit symptoms such as developmental delays, cognitive impairments, and motor deficits. Extended exposure may result in symptoms including memory impairment, tremors, and sensory disturbances. Mercury accumulates in the brain, liver, kidneys, and placenta. Inorganic mercury, derived from methyl mercury, may negatively impact kidney function over time. Prenatal exposure raises concerns due to its potential to induce brain impairment and developmental deficiencies in the fetus. Occupational exposure may result in mercury poisoning, particularly in sectors that employ mercury or its compounds. Acute exposure, even with limited contact, especially to dimethyl mercury, can result in fatal outcomes. Repeated exposure to mercury through contaminated food, water, or air may result in long-term health effects, including impairment of the immune and cardiovascular systems. Regular assessment of mercury concentrations in vulnerable populations is crucial for effective prevention.

5. CONCLUSION

Mercury accumulation in essential organs, including the brain, liver, kidneys, and placenta, is associated with significant health risks. The brain is susceptible to considerable cognitive impairments, motor challenges, and enduring neurological harm. The accumulation of mercury in the kidneys and liver poses significant risks to these organs; inorganic mercury, derived from methyl mercury, is especially responsible for long-term kidney damage. Mercury exposure during pregnancy poses significant risks, as it can lead to enduring neurological and developmental issues that impact fetal growth and brain function. Mercury poisoning poses a significant risk to workers in sectors such as mining, manufacturing, and healthcare that handle mercury or its derivatives. Acute exposure can be fatal even at low doses, particularly for highly toxic substances such as dimethyl mercury. Prolonged or recurrent exposure for workers may lead to chronic health issues, including irreversible damage to internal organs and the nervous system. Chronic exposure to mercury from contaminated food, water, or air can adversely affect brain development, cardiovascular health, and vulnerable systems in patients. Comprehensive monitoring of mercury exposure in high-risk populations is essential for effective management, alongside the implementation of strict regulations and measures to mitigate exposure. Mitigating the adverse impacts of mercury on public health necessitates safeguarding at-risk groups, including pregnant women and healthcare workers.

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Impact of chromium supplementation on human health and body composition

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ABSTRACT

Chromium(III) has been proposed to have a dietary or pharmacological position in changing frame composition and enhancing signs and symptoms of insulin resistance, kind II diabetes, and related conditions even though the mode of action of Cr(III) at a molecular degree has didn't be elucidated. This assessment info the cutting-edge reputation of research into Cr(III) supplementation. Clinical trials, meta-analyses and systematic critiques have didn't show clinically widespread results from Cr(III) supplementation on frame composition or symptoms of insulin resistance and related conditions in people and livestock. despite the fact that new Cr(III) supplements hold to appear inside the clinical literature, research have did not elucidate the mechanism of chromium action at a molecular level. Conflicting results on a position of transferrin in Cr(III) transport and detoxing have appeared. Cr(III) supplementation cannot presently be endorsed in people or livestock. further studies are required to probe the mechanism of Cr(III) movement in growing insulin sensitivity and glucose uptake in rodent fashions of insulin resistance and diabetes, with unique attention being became to a ability position of transferrin in Cr(III) transport

and cleansing.

Keywords: *Chromium, diabetes, insulin signalling, transferrin.*

INTRODUCTION:

In organic environments, two distinct forms of chromium (Cr) are typically established. The first is Cr(VI), typically encountered in chromate or dichromate forms, while the second is Cr(III). Chromium(VI) complexes are widely recognized as toxic substances, mutagens, and carcinogenic agents, particularly when ingested. They exhibit thermodynamic instability, readily converting to chromium(III) compounds, which represent the stable form of chromium in the presence of water and air. Chromium in its trivalent form is present in an extensive array of food items, yet it remains largely unrecognized by the general populace. It is posited that optimal nutritive Cr(III) is derived from the utilization of a chrome sword device in food processing; this suggests that individuals adhering to a weight loss regimen consume considerably lower levels of Cr(III) compared to the cutting-edge diets prevalent in developed nations. Despite the presence of toxicity in certain shops and the positive aspects of various cultures, Cr(III) is evidently not harmful to organisms under any reasonable treatment. No advanced tolerable restriction for humans has been established due to the absence of compelling evidence regarding the toxicity of any examined substances thus far. This does not imply that there are no potentially harmful consequences associated with elevated levels of Cr(III). In the 1950s, Cr emerged as a significant trace element for mammals. However, the essence of chromium has been the subject of considerable debate, primarily due to foundational concepts, and recent research indicates that beneficial effects in rodents regarding insulin sensitivity and potentially lipid profiles, including LDL cholesterol and triglyceride levels, necessitate supranutritional, pharmacologically relevant doses of Cr(III) [Vincent JB, et.al 2019, Vincent JB, et.al 2017, Vincent JB, et.al 2018]. In human existence, no consistent remedy exists – correlations between chromium and beneficial health

outcomes have been established. In 2014, the European Food Protection Authority (EFSA) found no compelling evidence to support the notion that chromium is a significant element. They concluded that there is no evidence of health concerns related to chromium intake in healthy individuals and that the recommended intake levels for chromium are no longer deemed appropriate. The historical examination of beneficial and pharmacological investigations involving Cr(III) has recently been thoroughly reviewed.

DISCUSSION:

Body composition:

In the late 1980s, assertions emerged that Cr(III) supplementation, especially as Cr(III) picolinate, could result in reductions in body mass and enhancements in lean body mass. Despite swift challenges to these assertions, Cr(III) picolinate became a widely used nutritional supplement, with product sales surpassing half a billion dollars (US). In 1997, the Federal Trade Commission (FTC) of the United States mandated that companies cease marketing claims, such as that Cr(III) diminishes body fat, induces weight reduction without exercise, or enhances muscle mass, due to insufficient 'competent and reliable scientific evidence.' [Federal Trade Commission,2019] Lukaski [Lukaski HC, 2019] recently reviewed the effects of Cr(III) on body composition, concluding that 'the preeminence of experimental findings underscores that Cr(III) supplementation... is neither a substantial contributor to body mass reduction nor a promoter of body fat loss or muscle gain in adults.' A 2019 systematic review of trace element supplementation in exercise and athletic performance concluded that Cr(III) supplementation, in the majority of research, demonstrated no correlation with measures of anthropometric or body composition changes. In 2019, the inaugural meta-analysis of studies regarding the benefits of Cr(III) on body composition was published, exceeding five times the previous research. The variables analyzed included body mass, body fat percentage, BMI, and waist circumference. No statistically significant influence was detected regarding fat or fat characteristics on midriff

circumference. Despite the majority of studies indicating no effect from Cr(III) supplementation for each variable, the meta-analyses revealed statistically significant improvements in body mass loss (0.75 ± 0.30 kg), reduction in body fat (0.68 ± 0.64), and decrease in BMI (0.40 ± 0.26). Group analysis indicated that the effects on body mass reduction and body fat alteration were especially pronounced for participants undergoing supplementation for 12 weeks or less and receiving 400 mg of creatine per day or less; specifically, the minimal dosages for the briefest time. Despite being statistically significant, these results lack clinical significance and are constrained by the restricted number of trials, of which few were of high quality. Another weakness of the meta-analysis is that the errors in two of the major studies were not adequately assessed. Supplementation of Cr(III) in boluses of up to 1000 mg daily does not seem to exert any clinically significant impact on body mass and composition in healthy, overweight, or obese individuals, assuming such effect exists, and cannot be endorsed.

Human health and disease:

For several decades, Cr(III) has been suggested as an adjunct treatment for insulin resistance, type II diabetes, and related conditions. Rodent studies indicate that large, supranutritional doses of Cr(III) can enhance insulin sensitivity and may lead to beneficial alterations in LDL cholesterol, total cholesterol, and triglyceride levels in both healthy and disease model animals [Vincent JB, et.al 2019]. In humans, the results remain unclear, possibly due to the fact that the doses of Cr(III) administered have not matched those utilized in the most rigorous rodent studies. From 2001 to 2004, the US National Institutes of Health (NIH) conducted a program advertisement focused on 'Chromium as an adjuvant treatment for type II diabetes and blood glucose management.' Clinical trials funded under this advertisement did not replicate the results of earlier trials typically financed by nutraceutical companies that reported beneficial effects in humans. In 2005, the US Food and Drug Administration (FDA) issued a letter of enforcement discretion regarding a request for eight health claims related to Cr(III) from a nutraceutical company. The FDA permitted

a single health claim, which noted that 'the actuality of such a relationship between chromium picolinate and either insulin resistance or type II diabetes is largely uncertain' [Costello RB, et.al 2016]. Consequently, due to these factors and the increasing evidence indicating that chromium (Cr) is not an essential element for organisms, governmental support for Cr(III) nutritional or pharmacological research has largely been absent in the United States and Western Europe in recent years. Recent exploration of the effects of Cr(III) on human health has focused on meta-analyses (e.g., [Huang H et.al 2018, Heshmati J, et.al 2018]), although several clinical trials have also been conducted (e.g., [Jamilian M, et.al 2019, Farrokhian A, et.al 2019]). The quantity of meta-analyses examining the effects of Cr(III) on the symptoms of polycystic ovarian syndrome is approximately equal to the number of clinical trials conducted. The findings from these meta-analyses and trials are inconsistent [Costello RB et.al 2019 & Costello RB, et.al 2016]. The outcomes of the meta-analyses are contingent upon the inclusion criteria, especially regarding the quality of the clinical trials incorporated, as higher quality studies generally report no beneficial effects from Cr(III) supplementation. The situation has been effectively summarized by Costello and colleagues: 'Until adequately powered trials that control for issues of nutrient phrasings, bioavailability, background diets, and drug use provide further conclusive evidence of the efficacy of Cr(III) supplements, the current support for health professionals to recommend Cr(III) supplements for glycemic control in diabetes cases is insufficient.'[Costello RB, et.al 2016] In contrast to clinical trials that have administered 1 mg or less of Cr daily, findings from rodent studies suggest that adult humans should consume a minimum of 10 mg of Cr daily, potentially allowing for larger doses of Cr(III) in similar trials. The American Diabetes Association asserts that there is insufficient evidence to endorse the routine use of herbals and micronutrients, such as chromium, for improving glycemia in individuals with diabetes.

Chromium supplements:

Alongside the Cr(III) supplements that have predominantly been utilized in research over the past two decades—such

as Cr chloride, Cr picolinate, Cr nicotinate, Cr propionate, Cr-amended incentive, and Cr methionine—there remains a growing body of literature documenting the use of alternative Cr(III) compounds as nutritional supplements [Berenjian A, et.al 2018, Feng W, et.al 2018, Feng W, et.al 2018, Feng W, et.al 2019, Krol E, et.al 2019, Guo W-L, et.al 2019, Cui J-F, et.al 2019, Ye H, et.al 2019]. A multitude of these accoutrements remain insufficiently defined. Indeed, the compound that has garnered the most attention in recent publications, Cr malate, has been inaccurately characterized, leading to a plethora of inquiries regarding the studies conducted with it [Smart M, et.al 2018]. Notwithstanding certain assertions, none of these accessories seem to offer any advantages linked to their use beyond those initially reported for the more thoroughly examined composites. For illustration, CrCl3, Cr nicotinate, and Cr picolinate, the three most prevalent Cr(III) supplements for human consumption, seem to be absorbed to similar extents, resulting in comparable Cr(III) levels in bodily fluids [Vincent JB, et.al 2019]. There has been some debate in the field regarding the implicit toxicity of Cr picolinate [Vincent JB, et.al 2019]. Nevertheless, there seems to be a consensus that the supplement poses no danger when ingested orally, as it is likely to degrade within the gastrointestinal tract, thus functioning solely as a source of Cr(III). Nevertheless, the emulsion has been suggested to possess toxic and mutagenic properties towards cultured cells and potentially to organisms if it enters the bloodstream entirely [Vincent JB, et.al 2019]. A recent study has confirmed that Cr picolinate exhibits clastogenic and cytotoxic properties in a cure-dependent manner towards Chinese Hamster Ovary (CHO) cells, while Cr propionate does not demonstrate such effects [Jiang L, et.al 2018].

CONCLUSION:

Recent studies have reinforced determinations. In humans, chromium is likely not an essential element. Cr(III) supplementation does not have beneficial effects on body composition, and the boluses used to date show no positive impact on the symptoms of insulin resistance, type II diabetes, or related conditions in humans. Furthermore, the use of

Cr(III) supplementation cannot be recommended for ranch animals due to insufficient data on treatment and response relationships, despite the absence of a clear response being demonstrated. The molecular mechanism for the enhancement of insulin resistance in rodents due to pharmacological boluses of Cr(III) remains unresolved. Transferrin is implicated in the detoxification of Cr(III) and may play a role in its transport related to the pharmacological properties of Cr(III); however, further studies are required.

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Platinum-based agents in oncology and strategies for tumor suppression

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ABSTRACT

The discovery of the anticancer properties of platinum derivatives by Rosenberg represents a notable progression in the development of chemotherapeutic approaches for tumor management. In 1978, cisplatin was the first platinum-based drug to gain approval from the Food and Drug Administration for the treatment of testicular cancer, along with advanced ovarian and bladder cancer. Platinum-based drugs, including cisplatin, carboplatin, and oxaliplatin, are widely employed in chemotherapy for cancer treatment. Cisplatin is widely used in the treatment of solid tumors, such as those found in the bladder, ovarian, lung, gastric, breast, cervical, and head and neck areas. Platinum-based drugs engage with cancer cells through interactions with DNA, resulting in alterations to the double helix structure. This interaction interferes with the mechanisms of DNA transcription and replication, leading to the eventual induction of cell apoptosis. The effectiveness of anticancer treatment using platinum-based medications is regrettably limited by the development of resistance. The negative consequences linked to platinum-based medications include issues with selectivity, considerable systemic toxicity, and the emergence of drug resistance, in addition to nephrotoxicity, ototoxicity, myelosuppression, and peripheral neurotoxicity. As a result, to tackle the resistance and toxic effects, alternative platinum derivatives are utilized, taking

advantage of progress in nanotechnology and chemical synthesis. Nanodrugs that contain platinum, such as platinum nanoclusters, demonstrate novel anti-cancer mechanisms and are employed in the treatment of different cancer types.

INTRODUCTION

Rosenberg identified the anti-cancer properties of platinum derivatives (1-3). This discovery represents a significant advancement in the evolution of chemotherapeutic approaches for cancer treatment (5). Cisplatin, the pioneering platinum-based drug, received approval from the Food and Drug Administration for the treatment of testicular cancer, advanced ovarian cancer, and bladder cancer (4). Platinum-based agents like cisplatin, carboplatin, and oxaliplatin are extensively utilized in the chemotherapeutic treatment of cancer (3). Cisplatin is a platinum-based chemotherapeutic agent primarily utilized in the treatment of solid tumors, including those located in the bladder, ovaries, cervix, lungs, breasts, stomach, and head and neck. Cisplatin-based chemotherapy for testicular cancer is regarded as nearly curative (4). Cisplatin and carboplatin, both platinum derivatives, have gained worldwide clinical approval and are essential components in most chemotherapy protocols for cancer treatment (5). The efficacy of platinum-based anticancer therapies is constrained by the emergence of resistance. The results reveal a range of negative impacts, including diminished selectivity, heightened systemic toxicity, and the development of drug resistance. Cisplatin is associated with nephrotoxicity and ototoxicity, carboplatin is recognized for inducing myelosuppression, and oxaliplatin is connected to peripheral neurotoxicity (6). The toxicities impose considerable constraints on dosage and negatively impact the quality of life for those who have overcome cancer. To address resistance and reduce toxic effects, new platinum derivatives and contemporary application strategies have been developed. Recent advancements in nanotechnology and chemical synthesis have significantly enhanced the efficacy of platinum-based anti-cancer drugs in the treatment of cancer. Platinum-based nanodrugs, such as platinum nanoclusters, exhibit novel anti-cancer mechanisms and show

significant potential for application in targeted tumor therapy. Cisplatin, a first-generation platinum-based anti-cancer drug, was discovered in the late 1960s and received approval for cancer treatment in 1978 (29). Cisplatin shows therapeutic efficacy against various malignant tumors, including breast, ovarian, and colorectal cancers (18). Cisplatin is a compound that causes systemic toxicity in addition to its cytotoxic actions on tumor cells. The prolonged administration of cisplatin results in significant harm to normal tissues. Consequently, the advancement of the second-generation platinum chemotherapy agent carboplatin occurred. Carboplatin, characterized by its reduced toxicity, is appropriate for application as high-dose chemotherapy in managing aggressive tumors. The administration of cisplatin and carboplatin often leads to the development of drug resistance during the treatment process. Consequently, the formulation of the third generation platinum compound, oxaliplatin, was achieved. Oxaliplatin functions in a manner akin to cisplatin; however, it does not induce cross-resistance with either cisplatin or carboplatin (36-37). Oxaliplatin and cisplatin exhibit a synergistic effect and are extensively utilized in cancer treatment (38-40). Among the diverse range of anti-cancer platinum agents, multifunctional high-performance platinum nano cluster-based (Pt NC-based) nanodrugs, developed from various biocompatible materials leading to versatile designs, have garnered significant interest for targeted cancer therapy and drug delivery [22, 23]. In contrast to conventional first-line platinum-based chemotherapeutics, Pt NC-based nanodrugs demonstrated enhanced stability, superior water dispersibility and solubility in vitro, along with reduced systemic toxicity and improved biocompatibility in vivo [24-26]. The distinctive benefits of Pt NC-based nanodrugs, including controllable fabrication, biosafety, and anti-tumor efficacy, underscore their significant potential for application in cancer treatment [27].

Molecular Mechanism of Cisplatin

Platinum-based anti-cancer agents, including cisplatin, carboplatin, and oxaliplatin, are extensively utilized in cancer treatment. Cisplatin primarily impacts cancer cells

by interfering with their DNA replication and transcription processes. Cisplatin predominantly enters tumor cells via the copper transporter 1 (CTR1). Upon entering the tumor cell, the platinum complex undergoes an activation step, during which the chloro-ligand is replaced by water molecules or other small molecules containing sulfhydryl groups. The concentration of chloride ions within cells is approximately 4 mM, significantly lower than the concentration outside the cells, which is about 100 mM. The decreased concentration triggers the replacement of chloride ions and promotes the transformation into cationic hydrates, such as cis-[Pt(NH3)2Cl(OH2)]+ and cis-[Pt(NH3)2(OH2)2]2+ [34]. The hydration rate and reaction with ammonia for transplatin occur significantly faster than those of cisplatin. The complex is swiftly rendered inactive prior to reaching its designated target, owing to the considerable reactivity of transplatin. Following a sequence of chemical reactions in the cytoplasm, the activated platinum complex binds to DNA, resulting in the formation of intra- and inter-stranded crosslinks. The structure of DNA is altered, resulting in damage and obstructing the processes of replication and transcription [36, 37]. Cisplatin primarily engages with the N7 position of guanine, which is easily accessible in the major groove and is preferentially involved in platinating reactions. DNA damage can disrupt the cell cycle and trigger apoptosis in rapidly proliferating tumor cells [38, 39].

Pt Nanoparticles (PtNPs) as a New Pt Drug for Cancer Therapy

Platinum nanoparticles (PtNPs) find applications in the diagnosis and treatment of cancer, along with addressing multi-drug resistance in bacteria (Jabir et al., 2012). Nanotechnology is utilized to address bacterial antibiotic resistance and assist in tumor management (Taylor and Webster, 2011). PtNPs incorporate hydrophilic polymers that improve opsonization, while MNPs are employed in cancer treatment (Jabiretal., 2012). Platinum exhibits significant anti-cancer characteristics (Reedijk, 1987). PtNPs exhibit distinct mechanisms of action when contrasted with platinum-based pharmaceuticals, while demonstrating comparable

efficacy in anticancer activity. PtNPs demonstrate cytotoxic effects in cancer cells via passive diffusion or endocytosis, with their effectiveness influenced by size, concentration, and incubation duration. The cytotoxicity primarily results from the induction of strand breaks in chromosomal DNA (Porcel et al., 2010; Johnstone et al., 2016; Kutwin et al., 2017). DNA damage interferes with replication processes and triggers cell cycle arrest as well as apoptosis. Another mechanism of platinum nanoparticles (PtNPs) involves the inhibition of cellular metabolic activity, the generation of hydroxyl radicals, and the release of active free Pt2+ ions; this strategy is currently employed in radiotherapy. PtNPs exhibit antioxidant properties at specific concentrations (Asharani et al., 2010; Gehrke et al., 2011; Prasek et al., 2013). Furthermore, PtNPs have the potential to enhance the host's anti-tumor immune response by improving antigen presentation and activating T cells (Yu et al., 2022). PtNPs demonstrate significant photostability following multiphoton stimulation within cellular contexts, making them suitable for imaging and microscopy purposes (Botchway et al., 2008). López Ruiz et al. (2020a) developed a unique dendritic structure composed of silver (Ag) and platinum (Pt) nanoparticles. AgPtNPs demonstrated a notable inhibitory effect on drug-resistant pathogenic bacteria such as P. aeruginosa, E. coli, and S. aureus, exhibiting greater efficacy than silver NPs. PtNPs underwent coating with PA (PA/PtNPs) to enhance targeted delivery to bone. The investigation explored the combined effects of photothermal therapy (PTT) and the anticancer properties of PA in malignant bone cancers (Zhou et al., 2019). Phytic acid (PA) is a polyphosphorylated carbohydrate that is frequently encountered in fiber-rich human diets, particularly in legumes and cereals, and is found in most mammalian species (Wilson et al., 2013). This shows enhanced effectiveness in addressing malignant bone tumors. Platinum nanodrugs that employ nanocarriers have garnered significant interest because of their extended blood circulation duration, enhanced permeability and retention (EPR) effect, and ease of surface functionalization.

CONCLUSION

Traditional tumor chemotherapy utilizes chemotherapeutic agents that frequently result in significant negative impacts on normal cells and tissues [13]. Platinum-based anti-cancer agents are essential in clinical oncology, demonstrating significant effectiveness. Cisplatin and other first-line clinical platinum-based anti-cancer agents are established treatments that show efficacy against tumors through clearly defined molecular mechanisms. The negative consequences significantly restrict the use of platinum-based anticancer agents. Investigations have been conducted on modified Pt-based drugs to assess their potential in enhancing anti-cancer efficacy while minimizing systemic toxicity. Pt NC-based nanodrugs have garnered significant interest owing to their extended blood circulation duration, enhanced EPR effect, and ease of surface functionalization. Recent advancements in nanotechnology and nanoscience have enabled the development of platinum nanoclusters (Pt NCs), which play a vital role in the investigation of platinum-based drugs designed with specific structures to enhance therapeutic efficacy and minimize systemic toxicity [27]. Therapeutic drugs derived from platinum have been extensively studied for their ability to target and eliminate tumor cells. The use of these chemotherapeutic agents presents considerable difficulties, as their dose-dependent acute and chronic side effects stem from their non-selective impact on both tumor and healthy cells. Recent advancements in eco-friendly nanomaterials have significantly impacted various fields, such as energy applications, electronics, biomedical engineering, and drug delivery. Platinum nanoparticles (NPs) represent a category of metal NPs that exhibit numerous benefits, including anti-tumor and anti-bacterial characteristics, which render them useful in a range of pharmaceutical applications.

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Unlocking the potential of tio2 nanoparticles as anti-cancerous agent

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ABSTRACT

Titanium dioxide (TiO2) nanoparticles have surfaced as a promising instrument in the realm of oncology, largely because of their distinct physicochemical attributes, biocompatibility and their capacity to generate reactive oxygen species (ROS) under certain conditions. Recent breakthroughs in nanotechnology have unveiled the potential of TiO2 nanoparticles as effective anti-cancer agents: they facilitate targeted delivery, controlled drug release and increased cytotoxicity directed at cancer cells, while minimizing damage to healthy tissues. This review delves into the mechanisms that underlie the anti-cancer effects of TiO2 nanoparticles, encompassing photocatalysis, the induction of oxidative stress and their role in synergizing with traditional therapies. The anti-cancer mechanisms of TiO2 nanoparticles (which are composed of titanium dioxide) primarily involve the generation of reactive oxygen species (ROS) upon exposure to ultraviolet or visible light. This process induces oxidative stress, DNA damage and apoptosis in cancer cells. However, TiO2 nanoparticles possess the potential to disrupt mitochondrial function and modulate signaling pathways that are crucial for cancer progression. Surface modifications and functionalization have, therefore, significantly enhanced their selectivity and efficacy; this

allows for targeted delivery while minimizing off-target effects. Although TiO2 nanoparticles are acknowledged for their intrinsic anti-cancer properties, they offer significant potential across various applications (such as photodynamic therapy (PDT), drug delivery and bioimaging). When used in conjunction with chemotherapeutic agents, they demonstrate a synergistic effect, enhancing drug efficacy while concurrently reducing the necessary dosages. Recent advancements in nanotechnology have also facilitated their use as diagnostic tools, offering high-resolution imaging capabilities for early tumor detection. This versatility positions TiO2 nanoparticles as a valuable asset in the ongoing fight against cancer; however, further research is essential to fully leverage their capabilities.

Keywords: *nanoparticles, biocompatibility, reactive oxygen species (ROS) , cytotoxicity , photocatalysis, oxidative stress , apoptosis , photodynamic therapy (PDT) , drug delivery and bioimaging*.

INTRODUCTION:

Cancer stands as one of the most widespread afflictions globally, impacting millions of individuals across diverse forms. In light of the considerable progress achieved in medical research, the quest for a definitive cure or universally effective therapy for cancer continues to be a challenging endeavor. Recent advancements in nanotechnology have unveiled intriguing possibilities for the improvement of cancer treatment and diagnosis. Nanoparticles, characterized as minuscule entities measuring less than 100 nanometers, have garnered considerable interest in the realms of health sciences and medicine owing to their distinctive attributes. Their diminutive dimensions, extensive surface area, and capacity for interaction at both cellular and molecular levels render them exceptionally suitable for precise therapeutic applications. It is essential to recognize that certain nanoparticles could potentially exert negative impacts on various animal cells, thereby underscoring the need for meticulous design and comprehensive testing within clinical environments.

Scholars are employing nanoparticles in diverse facets of cancer treatment to enhance efficacy and precision. In photodynamic therapy (PDT) and sonodynamic therapy (SDT), nanoparticles function as sensitizing agents, augmenting the production of reactive oxygen species to selectively eradicate cancer cells. In the realm of computed tomography (CT) imaging and radiation therapy, nanoparticles function as enhancement agents, augmenting the contrast and precision of imaging or amplifying the radiation dose administered to the tumor, all while mitigating harm to adjacent healthy tissues. Furthermore, there is ongoing development of nanoparticles for dual-mode imaging and therapy, wherein they function concurrently as contrast agents and therapeutic instruments. Titanium dioxide nanoparticles (TiO2 NPs) stand out among the various types of nanoparticles due to their significant application in the medical field, particularly in cancer research. Titanium dioxide nanoparticles are employed in photodynamic therapy, sonodynamic therapy, and drug delivery systems owing to their compatibility with biological systems, photoreactive properties, and potential for functionalization aimed at the precise targeting of cancerous cells. The ongoing challenge of cancer as a global health issue underscores the imperative for the advancement of innovative therapeutics and treatment modalities. The advancements in nanotechnology, poised to transform cancer treatment, are set to be integral to the future landscape of oncology. Scholars are committed to enhancing nanoparticle-based methodologies to optimize their effectiveness while reducing potential hazards, with the ultimate goal of delivering more efficient and tailored cancer therapies. Nanotechnology, first introduced by Richard Feynman in 1959, stands as one of the most swiftly advancing fields of scientific exploration and technological progress worldwide. The utilization and deployment of nanoparticles are significantly oriented towards the domains of chemistry, energy, healthcare, and cosmetics (Santos et al., 2015). Nanoparticles can be composed of organic and inorganic components, including ferritins, liposomes, micelles, dendrimers, and nanoparticles endowed with magnetic properties, metals, and semiconductor nanoparticles, such as

oxides, nitrides, and sulfides, respectively (Nabi et al., 2019). Titania, an oxide of titanium, is classified as a nanoparticle within the realm of metal oxides, showcasing unique optical, thermal, electrical, and magnetic properties. Under standard conditions, TiO2 remains insoluble in water and exhibits exceptional stability, characterized by a notable refractive index of n = 2.4, which makes it an ideal candidate for application as a white pigment. Titanium dioxide is generally found in three distinct crystalline polymorphs: anatase, rutile, and brookite. Among these three polymorphic forms, anatase and rutile display comparable properties; nonetheless, rutile possesses a lower density and demonstrates enhanced resistance to corrosion. Furthermore, brookite is infrequently observed owing to its instability at high temperatures, resulting in its conversion into rutile (Vijayalakshmi and Rajendran, 2012). The notable applications of nano TiO2 encompass photocatalytic degradation and splitting, photovoltaic cells, electrochromic and electronic devices, along with sensing instruments. These applications have generated considerable interest and prompted extensive research and development efforts directed towards the synthesis of TiO2 nanoparticles (Irshad et al., 2020, Mollavali et al., 2018, Narkevica et al., 2017, Pant et al., 2019).

Properties of tio2 nanoparticles:

Titanium dioxide nanoparticles (TiO2 NPs) possess several distinctive properties that render them invaluable in diverse medical applications, particularly in cancer therapy. The following delineates the principal properties of TiO2 NPs:

1. **Photoreactivity**: TiO2 NPs demonstrate robust photocatalytic activity, particularly under ultraviolet (UV) light. This characteristic allows for the effective generation of reactive oxygen species (ROS) upon exposure to light, a mechanism leveraged in photodynamic therapy (PDT) to eradicate cancer cells.

2. **Biocompatibility**: TiO2 NPs are generally regarded as biocompatible, indicating their ability to interact with biological systems without eliciting significant adverse effects. This trait is essential for their application in medical

fields, encompassing drug delivery and cancer treatment.

3. **High Surface Area**: Owing to their nanoscale dimensions, TiO2 NPs feature a high surface area-to-volume ratio. This promotes efficient engagement with biological molecules and cells, thereby augmenting their efficacy in medical applications.

4. **Chemical Stability**: TiO2 NPs exhibit chemical stability and resilience against oxidation and corrosion. This trait assures the retention of their functionality over time, which is vital for enduring medical applications.

5. **Surface Functionalization**: The surfaces of TiO2 NPs can be readily modified with various chemical entities or biomolecules, facilitating targeted delivery to specific cells or tissues, including cancer cells.

6. **Antimicrobial Properties**: TiO2 NPs have been demonstrated to possess antimicrobial characteristics, assisting in the prevention of infections when employed in medical devices or implants.

7. **Optical Properties**: TiO2 NPs exhibit commendable optical attributes, including a high refractive index and substantial UV absorption. These characteristics enhance imaging modalities such as computed tomography (CT) scans.

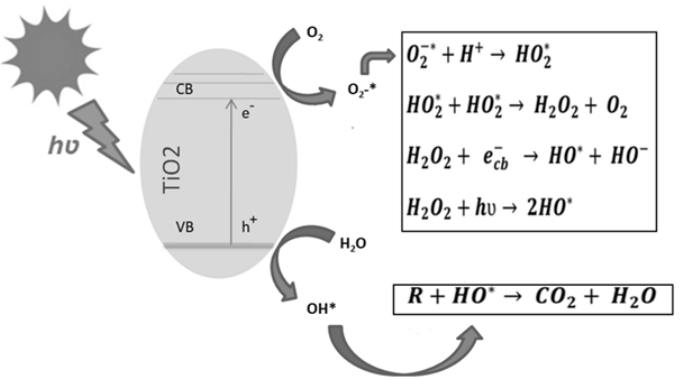
8. **Non-Toxicity**: Given appropriate dosages and controlled conditions, TiO2 NPs are relatively non-toxic, rendering them suitable for human applications. Nevertheless, their toxicity may vary based on size, shape, and surface enhancements.

These attributes establish TiO2 NPs as versatile instruments in cancer therapy, notably in amplifying the effectiveness of existing treatments like PDT and serving as vehicles in drug delivery systems.

Mechanisms of Anti-Cancer Action :

Cancer cells demonstrate an increased vulnerability to oxidative stress in contrast to normal cells, largely as a result of their modified metabolic pathways and the heightened

production of reactive oxygen species (ROS). This attribute makes them appropriate candidates for therapeutic strategies aimed at further augmenting ROS levels.



Titanium dioxide (TiO_2) nanoparticles demonstrate significant photocatalytic activity upon exposure to light, particularly ultraviolet (UV) or near-UV light, which results in the generation of various ROS, including hydroxyl radicals, superoxide anions, and hydrogen peroxide. These reactive species possess the ability to damage essential cellular components such as DNA, proteins, and lipids, thereby initiating apoptosis, or programmed cell death, in cancerous cells.

$$TiO_2 + h\upsilon \rightarrow e_{cb}^- + h_{vb}^+$$
$$h_{vb}^+ + H_2O \rightarrow H^+ + HO^*$$
$$e_{cb}^- + O_2 \rightarrow O_2^{-*}$$
$$O_2^{-*} + H^+ \rightarrow HO_2^*$$
$$HO_2^* + HO_2^* \rightarrow H_2O_2 + O_2$$
$$H_2O_2 + e_{cb}^- \rightarrow HO^* + HO^-$$
$$H_2O_2 + h\upsilon \rightarrow 2HO^*$$
$$R + HO^* \rightarrow CO_2 + H_2O$$

In the context of cancer treatment, TiO_2 nanoparticles are utilized in two forms: pure and functionalized. Pure TiO_2 nanoparticles rely on their inherent photocatalytic capabilities

to produce ROS, while functionalized TiO_2 nanoparticles are enhanced with specific molecules or coatings to improve targeting precision and ROS production efficiency. This modification enhances their selectivity towards cancer cells, thereby minimizing potential off-target effects on healthy cells.

In the context of photodynamic therapy (PDT), the interplay between light activation and TiO_2 nanoparticles enables a precise and localized increase in reactive oxygen species (ROS) levels, thereby effectively suppressing tumor proliferation. This approach minimizes damage to healthy tissues, as the light activation can be accurately directed at the tumor site, thereby enhancing the production of reactive oxygen species in the vicinity of the nanoparticles.

In cellular level, the role of titanium dioxide nanoparticles (TiO_2 NPs) on cellular metabolism and survival, emphasizing the role of reactive oxygen species (ROS) at different doses of TiO_2 NPs.

Mechanism of Action of Low Concentrations of Titanium Dioxide Nanoparticles (TiO_2 NPs)

1. **Moderate Reactive Oxygen Species (ROS) Generation**: At low concentrations, TiO_2 NPs generate a moderate quantity of reactive oxygen species (ROS) when subjected to light exposure. This induces a state of **mild oxidative stress** within cellular environments.

2. **Stimulation of Antioxidant Defense Systems**: The slight elevation of ROS levels activates the cell's **antioxidant defense mechanisms**. Crucial enzymes such as **superoxide dismutase (SOD)** are upregulated to neutralize ROS and avert cellular damage. SOD catalyzes the transformation of superoxide radicals into oxygen and hydrogen peroxide, which is less detrimental to cellular health.

3. **Optimized Mitochondrial Function**: In reaction to moderate oxidative stress, mitochondria adapt by enhancing their metabolic functions, specifically the **tricarboxylic acid (TCA) cycle**. This adaptation sustains cellular energy levels by ensuring a consistent supply of **adenosine triphosphate**

(ATP), the primary energy currency of the cell.

4. Cell Viability: The cell successfully maintains its metabolic equilibrium and energy production, enabling it to endure moderate oxidative stress. This resilience is conducive to continuing normal cellular function or facilitating recovery from mild stress conditions.

Mechanism of Action of High Concentrations of Titanium Dioxide Nanoparticles (TiO_2 NPs)

1. The elevated production of reactive oxygen species (ROS) is observed with high concentrations of TiO_2 nanoparticles, leading to a marked increase in ROS levels. This escalation results in significant oxidative stress, exceeding the cell's natural antioxidant defenses. Mitochondrial Impairment: The significant elevation in reactive oxygen species (ROS) causes harm to the mitochondria, leading to a disturbance in their membrane potential (MMP) and ultimately resulting in mitochondrial dysfunction. This dysfunction impedes the mitochondria's capacity to efficiently execute the TCA cycle, resulting in a significant decline in ATP production. 3. The release of cytochrome C occurs as a consequence of mitochondrial damage, leading to its presence in the cytoplasm. Cytochrome C is integral to the electron transport chain, and its release marks the commencement of mitochondrial-induced apoptosis, also known as the intrinsic apoptotic pathway. 4. The induction of the apoptotic pathway occurs when cytochrome C is released, leading to the activation of caspase 9, which in turn triggers caspase 3. Caspases serve as essential mediators within the apoptotic process. Caspase 9 serves as the initiator of the apoptotic signaling cascade, whereas caspase 3 carries out the cell death program through the cleavage of various cellular substrates. 5. The activation of the caspase cascade leads to apoptosis, a sophisticated mechanism of programmed cell death. Apoptosis is defined by a reduction in cell size, fragmentation of DNA, and the subsequent uptake by adjacent cells or phagocytes, which functions to mitigate inflammation. It can be inferred that:

1. Low concentrations of TiO_2 NPs augment the cell's ability to cope with oxidative stress, consequently improving cellular survival and metabolic stability.

2. Elevated levels of TiO_2 NPs, on the other hand, induce oxidative stress that surpasses the cellular capacity for management, leading to mitochondrial impairment, the initiation of apoptotic pathways, and ultimately, cell demise. The dual mechanism of action of TiO_2 NPs positions them as a significant contributor to cancer therapy, aiming to induce apoptosis in malignant cells while safeguarding the integrity of normal cells.

Potential Applications in Cancer Therapy: Titanium dioxide nanoparticles (NPs) find application across a wide spectrum of fields in medicine and life sciences. These nanoparticles act as vehicles for pharmaceuticals, especially anticancer agents, and operate as facilitators for photodynamic therapy (PDT) or photothermal therapy (PTT) in the management of solid tumors. Titanium dioxide nanoparticles have been adeptly utilized as carriers for a range of anticancer therapeutics, such as paclitaxel, doxorubicin, daunorubicin, temozolomide, and camptothecin. TiO_2 nanoparticles can be used to *directly target and kill cancer cells when activated by light.* This approach is particularly useful for *superficial cancers* (e.g., *skin cancer*) where light penetration is sufficient. Titanium dioxide nanoparticles (TiO_2 NPs) exhibit a diverse array of potential applications in cancer therapy, attributable to their unique properties.

Principal applications encompass:

1. Photodynamic Therapy (PDT): Titanium dioxide nanoparticles generate reactive oxygen species (ROS) when subjected to light, resulting in oxidative harm to malignant cells while safeguarding surrounding healthy tissue.

2. Sonodynamic Therapy (SDT): Analogous to Photodynamic Therapy (PDT), this methodology employs ultrasound to stimulate TiO_2 nanoparticles, thereby producing reactive oxygen species (ROS) to proficiently address deep-seated tumors.

3. Drug Delivery Systems: TiO_2 NPs enable the precise administration of anticancer pharmaceuticals to tumor locations, thereby improving therapeutic effectiveness and minimizing undesirable side effects.

4. The enhancement of radiation therapy through TiO_2 nanoparticles is noteworthy, as they significantly increase the production of reactive oxygen species during treatment, thereby augmenting its efficacy in targeting cancer cells.

5. CT Imaging: The incorporation of TiO_2 nanoparticles significantly improves tumor visibility in CT scans, thereby facilitating precise diagnosis and enabling targeted therapeutic interventions.

6. Dual-Mode Imaging and Therapy: TiO_2 NPs serve a dual purpose as both imaging agents and therapeutic tools, facilitating the seamless integration of diagnosis and treatment.

7. Induction of Apoptosis: TiO_2 NPs generate oxidative stress within cancer cells, thereby initiating programmed cell death (apoptosis) and inhibiting tumor proliferation, all while preserving the integrity of normal cells.

LIMITATION AND CHALLENGES:

While the potential applications of titanium dioxide nanoparticles (TiO_2 NPs) in cancer therapy are indeed promising, there are several noteworthy challenges that must be confronted:

1. Toxicity: Although TiO_2 NPs are typically regarded as biocompatible, there exists the potential for toxicity when administered at high doses or subjected to extended exposure.

2. Targeting Efficiency: The precise identification of cancer cells while safeguarding healthy tissues continues to present a significant challenge.

3. Regulatory Issues: The journey toward clinical application for TiO_2 NPs is fraught with rigorous approval processes, which elicit apprehensions regarding safety and environmental ramifications.

4. Aggregation: Within biological contexts, the aggregation of TiO_2 nanoparticles may occur, potentially reducing their efficacy and obstructing their capacity to traverse barriers, including the blood-brain barrier.

5. Light Penetration: The restricted capacity of light to infiltrate during photodynamic therapy diminishes the effectiveness of treatment for tumors situated deeper within tissues.

6. Scalability: The endeavor of producing these nanoparticles in substantial quantities while ensuring uniform properties poses a considerable challenge.

7. Absence of Standardization: The variability in nanoparticle properties presents significant challenges for their integration into clinical applications.

8. Immune Response: The aggregation of TiO_2 nanoparticles has the potential to elicit immune responses, consequently influencing their safety and therapeutic effectiveness.

Future prospect: TiO2 NPs are highly photoactive catalytic agent, with their affordable price, stability, it can be a hope for future cancer therapy. Despite having several limitations, these limitations underscore the necessity for further research to optimize TiO_2 NPs for cancer treatment.

CONCLUSION:

In summary, TiO_2 nanoparticles exhibit significant promise as anti-cancer agents, attributed to their photocatalytic properties, capacity to produce reactive oxygen species, and compatibility with biological systems. Despite the ongoing challenges related to safe delivery and precise activation, the progress in nanoparticle engineering and medical technology is poised to broaden their utilization in cancer treatment. Subsequent investigations will concentrate on overcoming existing constraints, examining the potential of combination therapies, and progressing towards clinical trials to validate TiO_2 nanoparticles as a credible alternative in the field of oncology.

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Optimization of Microbial Metabolite Production for Enhanced Plastic Degradation

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ABSTRACT

Plastics, particularly polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET), are extensively utilized yet remain in the environment due to their prolonged degradation rates. The global challenge of plastic waste accumulation has necessitated the exploration of effective and sustainable solutions. A promising approach involves utilizing microbial strains that can degrade plastic into less harmful compounds. These microbial strains are capable of metabolizing plastic components, thereby converting them into valuable metabolites. The optimization of metabolite production in these strains is essential for improving their biodegradation capabilities. This review examines strategies for optimizing metabolite production in microbial strains involved in plastic biodegradation, specifically highlighting *Gluconobacter oxydans*. The abstract of the paper summarizes the principal findings and importance of the research on the bioconversion of

ethylene glycol (EG) to glycolic acid (GA) utilizing the microorganism Gluconobacter oxydans. This document outlines the key points. Optimization of medium composition, enhancement of growth through specific additives, performance of bioreactors, implementation of an extended fed-batch strategy, and high process efficiency. The abstract provides a thorough overview of the research, highlighting the potential of Gluconobacter oxydans for glycolic acid production via optimized bioconversion processes. The main objective is to determine the optimal conditions for the bioconversion process by modifying operational variables, thus enhancing the efficiency and sustainability of GA production via microbial methods.

INTRODUCTION

Plastic pollution represents a significant global challenge. It is responsible for a broad range of environmental hazards and physiological toxicity. Plastic litter exerts significant physical effects on the environment. Nano plastics exhibit significant chemical effects and are associated with health hazards. Microplastic occupies a position within this spectrum. The degradation of plastic presents significant challenges owing to its durability and resistance to natural decomposition processes. Several methods have been developed or are under investigation to enhance the degradation of plastic. Mechanical recycling involves the collection, sorting, and melting of plastics to create new products. Chemical recycling includes processes such as depolymerization, pyrolysis, and gasification. Biodegradation processes include microbial degradation, enzymatic degradation, and composting for biodegradable plastics. The process of photodegradation, specifically through UV radiation. Thermal degradation, specifically through incineration and hydrothermal liquefaction. Additional advanced innovative technologies encompass Oxo-Biodegradable Plastics, supercritical Water, and Bioplastics. Each method presents advantages and disadvantages, and frequently, an integrated approach is necessary for the effective management of plastic waste. The primary objective is to establish processes that are environmentally sustainable and

economically feasible. (Sulajärvi et al., 2019) (Santos et al, 2024) The microbial biodegradation process can completely degrade plastic monomers and is sustainable; however, it is not yet applicable at the industrial level, unlike other methods that may not fully degrade plastic or may generate toxic byproducts. Microorganisms capable of degrading plastic monomers include Ideonella sakaiensis, Pseudomonas putida, Aspergillus niger, Rhodococcus ruber, and Gluconobacter oxydans, among others. Ethylene glycol (EG) can be synthesized from plastic waste via glycolysis, a chemical recycling technique specifically applicable to polyethylene terephthalate (PET) plastics. Glycolic acid (GA), a small molecule belonging to the alpha-hydroxy acid (AHA) group, is extensively utilized in diverse industries such as food, pharmaceuticals, textiles, and manufacturing. GA is conventionally synthesized from petrochemical sources via the catalytic reaction of formaldehyde with synthesis gas. There is increasing interest in sustainable production methods, particularly the use of microorganisms to convert ethylene glycol (EG), which can be derived from bioethanol, glycerol, or cellulosic biomass. (Santos et al, 2024)(Sulajärvi et al., 2019) Studies indicate that specific yeasts and acetic bacteria, including Gluconobacter oxydans, are capable of oxidizing EG to generate GA. Synthetic metabolic pathways utilizing genetically modified microorganisms such as Escherichia coli and Saccharomyces cerevisiae are being investigated for the efficient production of GA from simpler carbon sources, including glucose. (Zhang et al., 2022) A study aimed to optimize the bioconversion process of EG to GA utilizing Gluconobacter oxydans. The study examined critical operational variables such as bioreactor aeration, process temperature, pH, and cell concentration to enhance the efficiency, productivity, and yield of GA. (Zhou et al., 2021)

LITERATURE REVIEW

Glycolic acid (GA) is widely used across various industries, including food, textiles, pharmaceuticals, and manufacturing. It serves multiple roles, such as a dyeing agent in food, a skincare component in pharmaceuticals, and a tanner in

textiles. Additionally, it is involved in producing disposable packaging, emulsion polymers, and industrial cleaners, highlighting its versatility and importance in industrial applications.

Traditionally, GA is produced from petrochemical resources through chemical transformations involving toxic formaldehyde. However, there is a growing interest in bioproduction methods that utilize microorganisms, which offer a more sustainable alternative. The bioconversion of ethylene glycol (EG) to GA is particularly promising, as it can be derived from renewable sources like bioethanol and glycerol, making the process more environmentally friendly.

Various microorganisms, including naturally occurring yeasts and acetic bacteria, have been studied for their ability to oxidize EG to produce GA. This includes both wild-type strains and genetically modified organisms, which can utilize simpler carbon sources like glucose for enhanced production. The research indicates that optimizing microbial pathways can lead to more efficient GA production.

The study employs response surface methodology to optimize production levels while minimizing costs. This approach is crucial for enhancing the efficiency of the bioconversion process, focusing on variables such as aeration, temperature, pH, and cell concentration. The optimization of growth media, particularly the use of yeast extract and peptone, has shown significant improvements in cell growth and bioconversion yields.

The research highlights the effectiveness of different bioreactor configurations, such as stirred tank and bubble column bioreactors, in achieving high concentrations of GA. The extended fed-batch strategy demonstrated remarkable results, achieving a GA concentration of 94.2 g/L, showcasing the potential for industrial-scale applications. (Santos et al., 2024) (Zhou et al., 2021) (Jem & Tan, 2020)

The findings emphasize the importance of developing sustainable and economically viable methods for GA production. The study addresses technical challenges in scaling up the bioconversion process from laboratory to industrial

levels, aiming to create a more sustainable production pathway that incorporates renewable resources into the existing petrochemical framework. This literature survey underscores the significance of optimizing bioconversion processes and the potential of using microorganisms for sustainable glycolic acid production, paving the way for future research and industrial applications. (Santos et al, 2024)

The paper provides a comprehensive overview of the production methods for glycolic acid (GA) and ethylene glycol (EG), focusing on biotechnological approaches. GA is a versatile compound used in various industries, including textiles, food, pharmaceuticals, and cleaning agents. Its market has been growing, due to increased demand in cosmetic products and cleaning agents. Traditionally, GA is produced chemically from petrochemical resources, primarily through carbonylation of formaldehyde. However, biotechnological methods using microorganisms like Alcaligenes sp. and *Gluconobacter oxydans* have shown promise, with significant yields reported. EG is predominantly produced through the hydration of ethylene oxide. The paper highlights the potential for biotechnological production from renewable resources, although current methods are still reliant on chemical processes.

The paper discusses various metabolic engineering strategies employed to enhance the production of GA and EG using genetically modified microorganisms such as E. coli and yeast. These strategies have led to improved yields from sugars, although challenges remain in achieving commercially viable production levels. The paper identifies several challenges in the biotechnological production of GA and EG, including low production titers and the need for efficient metabolic pathways. It emphasizes the importance of balancing enzyme activities and metabolic fluxes to improve yields. The authors suggest that future research should focus on developing metabolic routes that utilize various cheap and renewable feedstocks, including agricultural waste. They also highlight the need for pathways that can produce both GA and EG at high yields. This literature survey encapsulates the current state of research on the biotechnological production of GA

and EG, outlining both the advancements made and the challenges that lie ahead in this field. (Sulajärvi et al., 2019). Glycolic acid (GA) is recognized for its diverse applications, particularly in cosmetics, packaging, and medical fields due to its properties as a bifunctional α-hydroxy acid. It is used in products for exfoliation and moisturizing, and in its polymeric form, polyglycolic acid (PGA), it serves as an effective packaging material and drug delivery substrate. Traditional methods for GA production involve chemical reactions that raise concerns about petroleum resource depletion and by-product formation. This has led to increased interest in microbial production methods utilizing renewable resources, particularly through engineered microorganisms that modify genes involved in the glyoxylate bypass. Recent studies have focused on constructing novel pathways for GA production in E. coli. These include the Dahms pathway and the glyoxylate bypass, which have been optimized to enhance GA yields. The research highlights the importance of overexpressing specific genes, such as lactaldehyde dehydrogenase (AldA) and glyoxylate reductase (YcdW), to improve production efficiency. The literature indicates that successful GA production in E. coli often involves gene knockouts and overexpression strategies. For instance, the deletion of the glycolate dehydrogenase gene (glcD) and the construction of strains like X17ld have been shown to significantly enhance GA production from D-xylose.

The regulation of the glyoxylate bypass is complex, involving transcriptional and protein-level controls. The aceBAK operon, which is crucial for the glyoxylate bypass, is activated under specific conditions, such as the presence of acetate or fatty acids. This regulation is sensitive to anaerobic conditions, which can impact GA production. The paper reports that the engineered E. coli strain GA19 achieved a notable production level of 4.57 g/L GA with a yield of 0.46 g/g from D-xylose, marking a significant advancement in microbial GA production through the Dahms pathway. This literature survey underscores the ongoing efforts to optimize microbial pathways for GA production, highlighting the importance of genetic engineering and metabolic regulation in achieving higher yields. (Cabulong et al., 2018). Prior

research indicates that incorporating organic nitrogen sources and reducing peptone concentrations can markedly enhance bacterial growth. The current study substantiates this finding by identifying yeast extract and peptone as the most effective nitrogen sources for Gluconobacter oxydans. The combination of mannitol with YE and PEP results in increased yields. Mannitol and glycerol demonstrate potential for industrial applications. (Santos et al, 2024) (Zahid et al., 2019) (Li et al., 2021).

CONCLUSION

The assays conducted for optimizing the composition of growth media demonstrated favorable outcomes, resulting in enhanced bacterial biomass and improved bioconversion yields of ethylene glycol (EG) to glycolic acid (GA). This suggests that the appropriate media composition is essential for improving microbial productivity. The research revealed that variables including cell concentration, aeration, and medium pH have a substantial impact on glycolic acid production in bioreactors. Managing these parameters is crucial for optimizing process yields, as they have a direct impact on the enzymes involved in glycolic acid synthesis. The bioconversion of ethylene glycol into glycolic acid by Gluconobacter oxydans indicates a promising avenue for industrial production. The findings indicate that this organic acid, currently in high demand, has the potential for sustainable production. However, there are notable challenges associated with scaling the process from bench to industrial levels. The research identifies specific technical limitations that impede the transition from laboratory-scale to industrial-scale production. This work enhances the development of a more sustainable and cost-effective approach to glycolic acid production, which is essential for its commercial success. The biotransformation process is associated with the petrochemical industry, utilizing ethylene glycol, a substrate predominantly sourced from petrochemical origins. The integration of renewability into the production process plays a crucial role in diminishing dependence on fossil feedstocks. The study emphasized that, although earlier research attained higher product concentrations, it failed to

consider the residual ethylene glycol present at the conclusion of the process. This oversight highlights the critical role of complete substrate utilization in enhancing operational efficiency and sustainability. The findings collectively highlight the efficacy of the optimization strategies utilized in the study and their possible implications for the sustainable production of glycolic acid.

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Therapeutic Applications of Heavy Metals: Anticancer Insight Into Ruthenium

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ABSTRACT

Heavy metals have revolutionized the field of oncology, with platinum-based drugs such as cisplatin serving as a foundational treatment. However, their severe side effects and resistance issues have necessitated the exploration of alternative metal-based therapies. Ruthenium has emerged as a promising candidate, offering distinct advantages over platinum due to its unique chemical properties, improved efficacy, and lower toxicity profile. this paper provides a comprehensive review of ruthenium base anticancer therapies, addressing key aspects that contribute to its potential as a therapeutic agent. The chemical characters of ruthenium, including its versatile oxidation states, coordination chemistry, and ligand exchange dynamics are explored to understand its stability and selectivity. The mechanism of action, such as DNA interaction, reactive oxygen species (ROS) generation, apoptosis induction and tumor microenvironment modulation, are critically analyzed. The efficacy of ruthenium complexes in preclinical and clinical studies is discussed, highlighting their ability to overcome multidrug resistance and reduce off-target effects. Recent advancements in clinical trials of ruthenium complexes are

also reviewed and offering insight into their transitional potential. This review emphasizes the growing importance of ruthenium on anticancer drug development, showcasing its role as a next generation therapeutic agents capable of addressing the limitation of existing treatments. By providing in depth understanding of its therapeutic potential, this paper aims to contribute to advancement of metallopharmateucals in cancer therapy.

Keywords: *ruthenium complexes, anticancer activity, therapeutic targets, clinical research*

INTRODUCTION

The organic ligand associated with the central metal in transition metals is crucial in scientific research, medicine, and pharmacy. Cancer has become a predominant cause of mortality globally. Chemotherapy is a commonly utilized treatment option. Nonetheless, its limited efficacy in certain instances results in a significant failure rate. Heavy metals are being investigated in chemotherapeutic drugs to enhance their efficacy. In 1965, Rosenberg discovered cisplatin, a platinum-based compound that revolutionized cancer chemotherapy and underscored the potential of heavy metals in oncology. Skoczynska A et al. (2023) This compound is utilized for the treatment of head, neck, bladder, and ovarian cancers. Skoczynska A et al. 2023 This drug has emerged as a primary anticancer agent owing to its significant effectiveness in addressing multiple cancer types. Nonetheless, despite its efficacy, it is linked to considerable adverse effects, such as cellular toxicity, peripheral neuropathy, and myelotoxicity [Lin k et al.2018]. These compounds are restricted to certain cancer types, underscoring the necessity for alternatives. The development of non-platinum metallic compounds has emerged as a priority in cancer research. Ruthenium is identified as a more promising option [Seva G et al. 2009].The mechanisms of action of ruthenium complexes differ from those of platinum and demonstrate greater efficacy. Over the last 30 years, numerous ruthenium complexes have been synthesized and subjected to clinical research to evaluate their effectiveness. This element is a transition metal belonging to the platinum group and shares the same chemical group

as iron, classified as a group 8 element.The complexes are recognized for their stability under physiological conditions. Ruthenium exhibits diverse oxidation states, including Ru(II), Ru(III), and Ru(IV). The elevated oxidation state exhibits reduced stability. The ruthenium ion typically exhibits hexa-coordination with an octahedral coordination geometry. Coordination can occur with various types of ligands. The geometry of these complexes can be modified by altering the ligands, facilitating the synthesis of various chiral ruthenium compounds with distinct properties. Ruthenium complexes, particularly Ru[II], are capable of ligand exchange reactions with biomolecules, enhancing their activity against disease targets. Ruthenium complexes demonstrate anticancer effects via a multifaceted mechanism of action, setting them apart from conventional platinum-based drugs. They accumulate selectively in tumor tissues through the enhanced permeability and retention effect and can be activated in the hypoxic and acidic environments of tumors, thereby minimizing damage to normal cells. This selectivity may result from ruthenium's capacity to imitate iron, which is significant for cancer cells due to their higher number of transferrin receptors, reflecting an increased demand for iron. This facilitates the more efficient delivery of ruthenium to cancer cells. The primary mechanism involves binding to DNA via intercalation, groove binding, or covalent attachment, which disrupts replication and transcription, ultimately resulting in apoptosis. Ruthenium complexes also produce reactive oxygen species (ROS), leading to oxidative stress, mitochondrial dysfunction, and subsequent cell death. They inhibit metastasis by targeting matrix metalloproteinases (MMPs), blocking angiogenesis, and interfering with cytoskeletal dynamics. Ruthenium complexes, in contrast to platinum drugs, can modulate essential signaling pathways, disrupt proteins such as heat shock proteins (HSPs), and enhance the immune response through the induction of immunogenic cell death. The diverse mechanisms of ruthenium complexes, along with their reduced toxicity and capacity to overcome drug resistance, position them as a promising alternative in cancer therapy.

DISCUSSION

Chemical Properties of Ruthenium:

Ligand exchange:

Ruthenium primarily engages in ligand exchange via substitution reactions, which are influenced by its coordination chemistry and the surrounding environment. The exchange is governed by the thermodynamic stability of the incoming ligand and the kinetics of the substitution reaction, facilitating selective interaction with cells. Ligands coordinated to a ruthenium center are substituted by other molecules from the surrounding environment [Bloemik K J et al. 1996]. The initiation of this ligand exchange is influenced by environmental factors such as pH, redox conditions, and the presence of biomolecules. This property is essential for cancer therapy, as ruthenium complexes can maintain stability in healthy tissues while undergoing selective activation in the tumor microenvironment, where lower pH and higher concentrations of reducing agents facilitate ligand exchange. Yamada H et al. (2001) This selective activation enhances the therapeutic targeting of cancer cells, minimizes side effects on healthy tissues, and facilitates the interaction of ruthenium complexes with molecules such as DNA, proteins, and enzymes [Reejdk J, 2003] [Yamada H et al. (2001)].

Oxidation state:

Ruthenium can exist in multiple oxidation states, typically Ru(II), Ru(III), Ru(IV).the reducing oxidation state is more stable. And under physiological conditions it form stable structures with six ligands in an octahedral shape. Unlike platinum drugs, which tightly bind DNA, ruthenium's geometry often prevents such binding, making it act differently in cells [Seva G et al.1995]

The cancer cell creates a different environment than normal cells. Due to have a reductive environment and reductive agent like glutathione and a lower pH, this initiate the reduction from Ru(III) to Ru(II). Scientist can take advantage of this by designing ruthenium drug as Ru(III) complexes (inactive prodrugs). Once inside the tumor, the reducing environment

activates them by converting Ru(III) to the active Ru(II) form. This allows the drugs to target cancer cells specifically. If the active Ru(II) complex moves to areas with more oxygen ,(like in normal cells), it can revert to its inert Ru(II) form, reducing damage to healthy cells. That make ruthenium -based drugs highly selective and less toxic. [Antonarakis E.S. et al. 2010]

Iron mimicking:

Iron is essential for nearly all living cells due to its significant role in crucial biological processes, including oxygen transport and DNA synthesis. Specialized proteins in the human body, such as transferrin and albumin, bind to iron to maintain its solubility in blood and facilitate transport and DNA synthesis. Specialized proteins in the human body, such as transferrin and albumin, bind to iron, maintaining its solubility in blood and facilitating its transport to necessary sites. These proteins facilitate the efficient delivery of iron, particularly to rapidly proliferating cells that require substantial iron to support their accelerated growth. Ruthenium, a transition metal, exhibits a distinctive capacity to bind to proteins similar to iron, especially transferrin. This occurs due to the similarity in chemical properties between ruthenium and iron, specifically in terms of charge and size. Consequently, ruthenium can associate with transferrin and gain entry into cells via receptors. Cancer cells exhibit an increased expression of transferrin receptors, facilitating enhanced iron uptake. Administration of ruthenium results in the uptake of the metal by these receptors, causing a significantly greater accumulation in cancer cells relative to normal cells.Research indicates that the concentration of ruthenium in cancer cells may be 2 to 12 times greater than in healthy cells [Seva G. et al. 2000].Furthermore, ruthenium can penetrate cells via alternative mechanisms independent of transferrin, enhancing its versatility. The selective targeting of cancer cells by ruthenium minimizes its impact on healthy cells, potentially reducing the systemic toxicity commonly associated with other treatments, such as platinum-based drugs. Antonarakis, E.S., et al. (2010)

Redox activity:

As ruthenium exhibit many oxidation states, its redox reactivity is crucial for anticancer therapy. In the reducing environment of cancer cells, ruthenium complex is reduced to ruthenium (III) to ruthenium (II) by intracellular reducing agents like glutathione and ascorbic acid, abundant in cancer cells. The redox flexibility allows ruthenium to generate ROS such as superoxide ions this superoxide ions undergoes further reaction, such as dismutation to form hydrogen peroxide (H_2O_2), then it generate hydroxyl radicals, within cancer cells causing oxidative stress that damage DNA, protein and induce apoptosis .

Stability and reactivity:

Ruthenium complexes are generally stable under physiological conditions, releasing hydrolysis and degradation. Their stability can be enhanced by selecting ancillary ligands, ensuring controlled reactivity in biological environment.

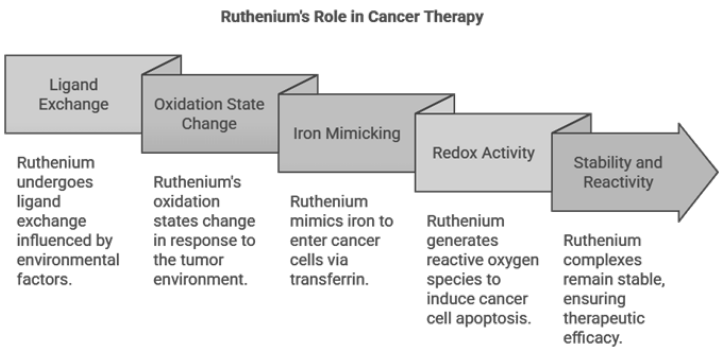

Fig 1: Chemical properties of ruthenium

Mechanism Of Action:

The ruthenium complexes have a diverse effect on human body. due to its coordination chemistry, stability and reactivity, it can target multiple sites and shows a diverse

mechanism for its antitumor properties. The Ru complexes some time bind directly with DNA and may cause DNA damage, and sometime it inhibits the enzyme responsible for DNA replication. it can also be effective deep down to the organelles and can cause cell death.

Binding with the DNA:

Ruthenium complexes demonstrate a variety of mechanisms of action against cancer, targeting DNA via different binding models, including electrostatic attraction, intercalative binding, and groove binding. This interaction interferes with replication and transcription, resulting in DNA damage and the inhibition of associated enzymes. Ru(II) complexes exhibit a preference for binding at the N7 site of guanine and the N3 site of thymidine. In contrast, Ru(III) complexes demonstrate a greater affinity for the phosphate backbone, attributed to electrostatic interactions, while also engaging with the N7 site of guanine.[Zeng W. et al., 2019]This interaction resulted in the unwinding and lengthening of the DNA helix, as confirmed by UV-Vis spectroscopy, viscosity studies, and fluorescence spectrometry.[Huang et al., 2016] Some Ru complexes, such as dinuclear and polynuclear Ru(II) polypyridyl compounds, exhibit stable binding to the G-quadruplex structure of telomere DNA, thereby inhibiting telomerase activity and obstructing tumor cell immortality.[Hiyama H. et al., 1995]Ruthenium complexes, including Ru(III)-PTA and Ru(II)-arene compounds, induce DNA damage through the formation of adducts with guanine residues, leading to apoptosis in cancer cells. Some compounds exhibit synergistic effects with ionizing radiation, demonstrating greater potency than traditional drugs such as oxaliplatin.

Inhibiting Topoisomerase Activity:

Topoisomerase II is essential for addressing topological challenges encountered during DNA replication, transcription, chromatin remodeling, and recombination by creating single- or double-strand breaks in DNA [Kurzwernhart A. et al. 2012]. Ruthenium-based complexes exhibit significant topoisomerase inhibitory activity, particularly against Topo II, disrupting its catalytic cycle and inducing apoptosis in cancer

cells by inhibiting DNA replication. Intense half-sandwich Ru-arene complexes with thiosemicarbazones function as Topo II inhibitors. Certain compounds demonstrate moderate anti-proliferative activity. and some have the higher potency than etoposide (ETP) . To overcome limitations in single target inhibition, researchers have developed ruthenium complexes exhibiting dual inhibitory properties against Topo I and Topo II, resulting in significant suppression of tumor cell proliferation. Multi-target ruthenium complexes exhibiting dual effects on G4-DNA and Topo have been synthesized, as reported by Liao et al. (2015), demonstrating synergistic apoptotic effects on tumor cells and efficacy against drug-resistant strains.

Targeting cell organelles:

In mitochondria:

Mitochondria serve as essential energy hubs within the cell, modulating apoptosis via pathways that involve mitochondrial membrane potential and related signaling proteins. Ruthenium complexes exhibit a strong affinity for mitochondria, leading to disruption of mitochondrial function upon accumulation [Wang X. et al. 2013]. Ruthenium complexes rapidly diminish the mitochondrial membrane potential through the generation of reactive oxygen species (ROS). It can occasionally disrupt the electron transport chain, particularly at complexes I, II, and III. Ruthenium disrupts electron flow, leading to a buildup of protons and electrons, which destabilizes the gradient necessary for membrane potential. This interference can result in electron leakage and mitochondrial dysfunction. This disruption impairs mitochondrial ATP production, resulting in an energy crisis within tumor cells. Dysfunctional mitochondria release cytochrome C into the cytosol, activating pro-apoptotic proteins such as Bcl2. This initiates a cascade reaction of caspase enzymes, which dismantle the cell in a regulated process of apoptosis (Sano R. et al., 2013). An efficient Ru-based compound [Ru(dppz)2(CppH)]2+ accumulates in mitochondria and demonstrates cytotoxic effects against cisplatin-resistant cancer cells, indicating ruthenium's ability to circumvent traditional resistance mechanisms.

In Endoplasmic Reticulum:

The ER plays a vital role in protein folding, lipid synthesis, and calcium storage. In cancer, it is often involved in promoting cell survival under stress. Ruthenium complexes can target the ER, leading to oxidative stress. Ruthenium complexes can cause the accumulation of reactive oxygen species within the ER, leading to the oxidative stress. The accumulation of misfolded proteins and other disruption in the ER activates stress response that led to apoptosis. Ruthenium –induced ERS involves activation of caspases, which triggers programmed cell death. By targeting the ER, ruthenium complexes can reduce autophagy and inhibit mechanism that typically allow tumor cells to evade drugs. [Gill M R.et al.2009] [Sano R.et al.2013]

In lysosomes:

Ru complexes can localize in lysosomes disrupting their structure and function. They increase lysosomal membrane permeability by the generation of ROS by fenton reaction leading to the production of hydroxyl radicals. This can cause lipid peroxidation of the lysosomal membrane, disrupting its integrity. These complexes also can directly interact with lysosomal membrane components, such as phospholipids and lysome-associated membrane proteins (LAMPs). Some ruthenium complexes down regulate LAMPs expression, which is crucial for maintaining lysosomal activity. Reduced LAMP level destabilizes the membrane and leading to increased permeability. This increase membrane permeability can cause the release of digestive enzyme like (cathepsin B) into the cytoplasm. The release of his enzyme damages cellular components, leading to lysosomal mediated cell death. [Sano R.et al.2012] [Lin K.et al.2018]

Targeting Cell Membranes:

The cell membrane acts as a both protective barrier and a direct target for Ru complexes. Ru complexes alter the membrane's permeability, allowing cellular components to leak out, leading cell death. Some Ru complexes emit red phosphorescence and produce singlet oxygen upon activation. This destroys the cell and mitochondrial membrane simultaneously, enhancing

cytotoxicity. [Lin K.et al.2018]

Ruthenium Based Anti-Cancer Drugs:

NAMI –A

NAMI-A, or [lmH][trans-Rucl4(DMSO)(lm)], is a Ru(III) complex featuring DMSO and imidazole coordinated to a ruthenium center. Preclinical studies have shown that NAMI-A exhibits significant antitumor effects, particularly in its anti-metastatic properties. The mode of action of NAMI-A includes interaction with cell cycle regulation, resulting in a temporary accumulation of cells in the G2/M phase. Inhibition of matrix metalloproteinase. Promotion of an extracellular matrix surrounding the tumor vasculature prevents tumor cells from invading adjacent tissues and blood vessels, while also coordinating with nucleic acids, thereby directly impacting tumor cell DNA. Animal studies indicate that more frequent, smaller doses of NAMI-a exhibit a stronger anti-metastatic effect. Importantly, its effect appears to be independent of the type of primary tumor. NAMI A has demonstrated efficacy in both preventing the formation of metastases and inhibiting the growth of established metastases. Preclinical studies using murine models demonstrated the selective efficacy of NAMI A against lung metastases originating from diverse primary tumors. The treatment reduces the weight of lung metastases more significantly than their quantity. The absence of higher concentrations of NAMI A in the lung compared to other tissues suggests that NAMI A selectively interferes with the growth of established lung metastases. Toxicity studies in mice and dogs demonstrated an acceptable safety profile, with a half-life of approximately 18 hours. Toxicity occurs at doses exceeding 50 mg/kg/day; however, any adverse effects were reversible within three weeks following the cessation of treatment. NAMI A was the inaugural ruthenium-based drug to advance to human clinical trials. A phase one pharmacokinetic study commenced in 1999 and was published in 2004. The study involved 24 patients with various metastatic solid tumors. Including collateral, lung, melanoma, ovarian, and pancreatic cancers that exhibited resistance to conventional therapies. Patients received NAMI A through a three-hour intravenous infusion daily for five days, repeated every three weeks, in

accordance with a dose escalation protocol. The dose range was 2.4 mg/m²/day to 500 mg/m²/day. At a dosage of 400 mg/m²/day, the patient developed painful blisters on the hands and feet, which persisted at the 500 mg/m²/day dosage. This condition is well recognized as a dose-limiting toxicity.The Phase II trials indicate that a dosage of 300 mg/m²/day is optimal, as it did not result in blisters, thereby enhancing tolerability. Additional side effects encompass anaemia, lymphopenia, fatigue, anorexia, stomatitis, peripheral oedema, nausea, and phlebitis at the infusion site. Occasionally, patients experienced hypersensitivity reactions that were resolved with dexamethasone. The study indicates that some patients were evaluable for tumor responses. A patient with a history of extensive treatment exhibited stable disease for 21 weeks in the context of progressive metastatic non-small cell lung cancer. The remaining patients exhibit disease progression. A phase II study evaluating NAMI-A has not been performed. [Antonarakis E. S. et al., 2010]

KP1019

KP1019 is a ruthenium-based compound demonstrating potential efficacy in cancer therapy. It induces apoptosis in cancer cells, specifically targeting collateral cancer cells. This occurs via mechanisms such as disrupting cellular energy production, damaging mitochondria, and activating specific proteins that induce apoptosis. The mechanism of action does not appear to depend on DNA damage as other cancer drugs do; however, it may induce some DNA damage indirectly via the generation of reactive oxygen species. Research involving mice demonstrated that KP1019 exhibited significant efficacy against colon cancer, including tumors that were resistant to conventional therapies such as 5-fluorouracil and cisplatin. The treatment resulted in significant tumor reduction, accompanied by mild side effects such as increased red blood cell production. In clinical trials involving humans, KP1019 was administered intravenously to patients with advanced solid tumors, including colorectal, endometrial, melanoma, and bladder cancers. The maximum dose evaluated was 600 mg

administered biweekly, which demonstrated good tolerance with only mild adverse effects. The drug exhibited slow absorption into the bloodstream, with a significant portion binding to proteins such as albumin. A minimal quantity was eliminated via the kidneys.In a trial, five out of six patients exhibited disease stabilization, with no progression of cells observed for a duration of 8 to 10 weeks. This suggests that KP1019 may have potential as a cancer treatment, and a phase II study is being planned to evaluate its efficacy in patients with advanced collateral cancer. Antonarakis, E. S., et al. (2010)

Other Ruthenium Complexes:

Polypyridyl-Ru complexes:

Researchers are investigating ruthenium-based complexes with polypyridyl ligands as potential anticancer agents due to their ability to bind to DNA and disrupt its function. Early studies indicate that certain ruthenium complexes, such as mer-[Ru(terpy)Cl3], exhibit greater cytotoxicity towards cancer cells compared to other drugs, primarily through the formation of DNA crosslinks. Modifications to these complexes, including the addition of various ligands and the combination of ruthenium with platinum, have been evaluated to improve DNA binding and enhance anticancer efficacy. Certain ruthenium complexes release nitric oxide upon light exposure, presenting potential applications in phototherapy. Despite their potential, these complexes have not yet commenced clinical trials. Ang, W. H., et al. (2006).

Arene PTA Ruthenium (II)(RAPTA) complexes:

The synthesis of RAPTA compounds occurs in two steps. Initially, a Birch reduction is employed to synthesize the desired arene, which subsequently reacts with hydrated Ru(III)Cl3 to yield a dimeric complex. In the second step, two equivalents of 1, 3, 5-triaza-7-phosphatricyclo [3.3.1.1] decane (PTA) are introduced to this complex to yield the final RAPTA compound. Alternatively, ligand exchange may be employed for arene ligands that are challenging to reduce via the Birch reaction, although this approach typically results in lower yields. The products exhibit stability in air,

thermodynamic stability, and solubility in both organic solvents and water. The RAPTA-C prototype has been pivotal in biological research, focusing on two primary approaches: the development of RAPTA as a selective anticancer agent and the exploration of its non-classical therapeutic potential via protein binding studies. More than 20 RAPTA complexes have been synthesized, encompassing derivatives with various ligands and metal fragments. Enhancing their applicability in pharmaceutical development. Ang, W. H., et al. (2006).

Ru complexes with nanoparticles:

Ruthenium based anticancer therapies faces many challenges due to its ideal balance of steric hindrance and lipopholicity is difficult. To address this, Nano drug delivery system offer significant advantages, including enhanced targeting through the EPR effect and active targeting via receptors receptor or enzyme modifications, reduced systemic toxicity, improved lipopholicity without structural changes, and the ability to deliver multiple therapeutic agents simultaneously. Encapsulation of Ru complexes such as selenium nanoparticles, gold nanomaterial and carbon nanotubes, improves targeting and delivery to tumor cells. Additionally, excipient free Nano drugs, composed primarily of active pharmaceutical ingredients with minimal additives, offers a unique approach where the matrix itself contributes to the therapeutic effect, distinguish them from traditional drug carriers. This integration of Ru complexes with advanced NDDS demonstrate great potential for enhancing antitumor activity. [Lu Y.et al.2022]

Using gold as a carrier for ruthenium complex:

The application of gold nanoparticles as carriers for ruthenium complexes in biomedical contexts, especially in cancer treatment, is highly effective. Gold nanoparticles exhibit various morphologies, including nanospheres, nanorods, nanocages, and nanoshells, and possess unique plasmon resonance properties that enable the absorption of near-infrared light and its conversion into heat for photothermal therapy. Initial research employed Ru polypyridine

complexes as stabilizers and coatings for AuNPs, investigating their photophysical and luminescent characteristics. Combining Ru complexes with gold nanoparticles resulted in enhanced luminescence, whereas their interaction with gold nanorods led to quenching of emissions. Further research has advanced the application of these conjugates in imaging, as they emit strong luminescence detectable through confocal laser microscopy and two-photon microscopy. Advanced design should enhance photothermal efficiency and stability, providing effective treatment in cellular and animal models. Recent studies have concentrated on the modification of gold nanoparticles (AuNPs) using ruthenium polypyridine complexes through sulfur-gold interactions. This complex demonstrated improved solubility, prolonged blood circulation, and enhanced memory permeability. This modification has enhanced therapeutic efficacy, particularly in collateral adenocarcinoma cells, and promoted apoptosis through improved cellular uptake facilitated by hydrophobic ligands and polymers [Lu Y.et al.2022]

Use of cationic liposome as a Nano carrier:

Cationic liposomes serve as effective organic carriers for the delivery of ruthenium complexes in cancer treatment. These liposomes effectively transport Ru complexes to tumor sites. In 2013, researchers developed an anti-tumor nanocarrier utilizing cationic DOTAP for the delivery of amphiphilic Ru complexes. The aggregates formed by mixing Ru complexes with phospholipid carriers at a 50/50 molar ratio exhibited 10 to 20 times greater activity compared to other compounds. In 2015, it was discovered that cholesterol complexes with DOTAP liposomes effectively prevented the degradation of Ru-based anti-tumor drugs. Recent developments involve the formulation of cationic Ru metal complexes with DOPE for the delivery of genetic material in gene therapy, thereby broadening the application of Ru complexes in biomedical fields [Lu Y.et al.2022]

Conclusion

Ruthenium-based compounds have emerged as a promising alternative to traditional platinum-based anticancer drugs

due to their low toxicity, organelle-specific targeting, and effectiveness against platinum-resistant tumors. The complexes demonstrate various mechanisms of action, including the disruption of mitochondrial function, induction of oxidative stress, and interference with lysosomal stability, all contributing to apoptosis in cancer cells. Their capacity to modulate lysosomal membrane permeability, decrease mitochondrial membrane potential, and induce endoplasmic reticulum stress highlights their multi-targeted therapeutic potential. Innovations in ligand design have led to Ruthenium complexes exhibiting dual inhibitory effects on topoisomerase, enhancing G4-DNA stabilization and photosensitizing capabilities, thereby broadening their application in targeted cancer therapy. Recent advancements in row ruthenium-based photodynamic therapy agents, utilizing their photophysical properties for selective tumor targeting, have enhanced the therapeutic application. Research on Ruthenium-based nanocarriers and drug delivery systems has shown enhanced cellular uptake and tumor specificity, thereby reducing off-target effects. Ruthenium complexes exhibiting dual targeting capabilities, which facilitate organelle-specific accumulation and address drug resistance pathways, present significant potential for overcoming challenges associated with chemotherapy. The exploration of Ruthenium complexes as multi-targeted agents has the potential to significantly advance cancer therapy. By integrating their inherent anti-cancer properties with advanced technologies like nanoparticle conjugation, gene delivery systems, and theragnostic, these compounds may attain enhanced therapeutic efficacy and precision. The integration of artificial intelligence in drug design and predictive modeling may accelerate the identification of novel ruthenium compounds with optimal activity profiles. Ongoing research and clinical trials indicate that ruthenium-based therapies are positioned to overcome limitations in current treatments, offering more effective, targeted, and patient-friendly solutions. Their versatility, along with advancements in nanotechnology and bioengineering, positions them at the forefront of next-generation anti-cancer strategies.

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Is Organic Food The Answer? Examining The Benefits And Pitfalls For Modern Living

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ABSTRACT

Having accessibility of high-quality food is becoming more difficult for everyone as the world's population increases. In India, the organic food manufacturing industry is only getting started. Consumers demand for organic food has grown domestically because of the rise in disposable income and increased health consciousness. Organic food refers to food that's made with using organic farming methods. Food which are environmentally friendly are grown without the use of synthetic chemicals like pesticides and fertilizers, and it doesn't include any type of GMOs. Organic food is preferred by consumers for a variety of reasons, including environmental, health, and animal welfare concerns, as well as perceptions of superior taste compared to conventional options. Organic agriculture is very much beneficial for the environment. Certified organic agricultural methods limit the use of synthetic fertilizers and pesticides, reducing chemical runoff and pollution of soils and waterways. According to the finest and most current research in systematic evaluations and meta-analyses, there are notable and nutritionally significant variations in the constituents of conventional and

organic foods. This includes elevated proportions of omega-3 fatty acids in dairy and meat products deemed organic, and additionally higher antioxidant levels in organic vegetables but lower levels of cadmium and pesticides. Overall, it seems that organic farming systems, similar to those who preceded earlier of sustainable agriculture, have previously demonstrated their capability to produce foods of superior quality; yet, there is currently not enough of scientific data on the health consequences of organic foods.

Keywords: *Organic food, consumer perception, health consciousness, genetically modified organisms (GMOs), organic farming systems.*

INTRODUCTION

The general public's conviction that food that has been produced organically is safer, healthier, and savours better than conventional food has played a significant influence in the advancement of the organic food industry. There are multiple motives that's why different demographic groups find organic food appealing. In this paper, we examine the information that is accessible regarding the connections between human health and farming systems (conventional vs. organic) (Axe et al. 2017).

The widespread use of traditional farming methods that align with organic agricultural practices in underdeveloped nations presents a significant opportunity to boost organic production. Out of the 143 million hectares of agricultural land in India (Varghese et al. 2016), only 5.2 million hectares (3.64%) are certified organic.

The Indian government launched the Paramparagat Krishi Vikas Yojana (PKVY) to encourage organic farming. Five lac farmers will benefit from the two lac hectors set aside for organic farming. In 179 nations worldwide, more over 50.9 million hectares including areas undergoing conversion were grown organically in 2015. The European Union's organic retail market grew by 107% between 2006 and 2015, reaching €27.1 billion (Helle Raun et al. 2017).

When considering the demand side of organic food markets, a number of factors influence customer decisions, such as product certification, perceived health benefits, and organic food pricing. The People have been digging at an increasing rate for organic products since they anticipate they provide better nutritional value and have a smaller environmental impact, driven by increased disposable incomes and a greater emphasis on wellness. According to a meta-analysis of 343 research on phytochemical composition, crops cultivated organically had 17% greater amounts of polyphenols and lower levels of pesticide and cadmium residues than crops grown conventionally. Numerous foods, specifically root vegetables and green nutritious vegetables, demonstrated to have a reduced nitrogen concentration when cultivated organically as in comparison to conventionally (Anne Lise et al. 2016). Compared to men, older age groups, persons of various political affiliations, and those with less education, research conducted in the United States has shown that women, young adults, liberals, and College graduates have a considerably greater chance of consistently purchasing organic food. Organic food may also be more appealing to adherents of other restricted diets. According to one research, disciples of vegetarian, vegan, or pescetarian diets consumed significantly more organic foods than omnivores. Organic agriculture has higher cost implications as a result of shorter yields and higher labour expenditures, resulting to strategic customer pricing. Since 2002, On a global scale purchases of organic food have increased by almost 170 percent., reaching over $63 billion in 2011(Stefan et al. 2017). The cost of organic items is generally twice as higher than that of comparable conventionally produced goods, ranging from 10% to 50%. However, the real scenario of organic food manufacturing is far more nuanced.

In light of contemporary living, this paper seeks to critically analyse the multifaceted nature of organic food, examining both its possible advantages and disadvantages. For the purpose to figure out whether or not organic food can actually be a sustainable and equitable alternative of the difficulties facing contemporary food systems, it will examine the environmental benefits and drawbacks of organic farming

practices, examine the scientific data regarding the dietary variations between conventional and organic foods, and evaluate the economic and social possibilities of widespread manufacture of organic food(Crystal Smith et al. 2012).

MATERIALS AND METHODS

A comprehensive investigation was carried out using PubMed, PubMed Central, Google, furthermore published research and overview paper from various countries. The primary objective of this review was on the environmental pollutants that were brought forth by the leather industries and the microbial bioremediation strategy to eliminate these pollutants. We removed statements of exposure that were too general and only looked at published data. Based on these inclusion requirements, we have contained data acquired from credible sources of relevant publications. Other than English, no other languages were considered in the study.

DISCUSSION

Chemical composition

Depending on the food group, organic and conventional alternatives differ in composition. According to an analysis published in February 2016, which published 15 research studies or meta-analyses examining the nutritional variations between conventional and organic products, 12 summarized those organic foods had enhanced vitamin C levels, total antioxidants, and total omega-3 fatty acids (Anne Lise et al. 2016). Due to variations in the tests conducted and the testing procedures, as well as the fact that the whims of agriculture impact the chemical makeup of food, these studies typically have confounding variables and are challenging to generalize. The chemical makeup of a particular food item is further influenced by the conditions of transportation and storage, a duration between harvest and inspection, and the handling of the food items afterwards the initial harvesting (Baranski et al. 2017).

Nutrients: A prevalent myth is that organic foods are higher in nutrients and are therefore nutritious than those developed in the conventional manner. However, because to varying

findings from field studies, specialist have not been 100% certain that this is the case.. According to reviews and meta-analyses, the amount of dry matter in organic fruits and vegetables is more severe, minerals (such as iron, magnesium, phosphorous, and zinc), vitamins C, and other bioactive substances like carotenoids and tocopherols, and lower levels of nitrate (Anne Lise et al. 2016). Alpha-tocopherol in beef and pork and the concentration of vitamin A (retinol) in beef turned out to be the same in the few studies that examined vitamin composition of meats. In contrast to conventional feed, which consists of soy, palm kernel cake, and cereals with lesser omega-3 content (Śmith-Spangler et al. 2012), organic livestock husbandry states that a significant portion of the feed for milk and dairy products be locally grown grass and clover, which are high in omega-3(Średnicka-Tober et al. 2016). As stated by systematic review and analysis, organic livestock had much amounts of good fats like omega-3 and omega-6 (Magkos et al. 2003) but lower levels of monounsaturated and saturated fat than conventional meat.

Nitrogen and phosphorus: When analysing organic to conventional, previous systematic evaluations have consistently revealed a higher phosphorus content (standardized mean difference) and a lower total nitrogen level (Magkos et al. 2003). One United Report has been examined that Arsenic levels in chicken might be lower that are organically nurtured when assessing environmental pollutants such heavy metals. However, given the variations in fertilization techniques and the essential role that N, P, and the N:P ratio play in plant development (Dangour et al. 2009), this could provide some credibility to other outcomes of the production system that have been seen.

Phytochemicals & polyphenols: During a meta-analysis of research on phytochemical composition, crops cultivated organically had 17% greater amounts of polyphenols and lower levels of pesticide and cadmium residues than crops grown conventionally. There are many limitations in research on the phytochemical makeup of organic crops, including the lack of standardized measurements, insufficient explanation of variability measures, selective or redundant data reporting,

publishing bias, and insufficiency of rigidness (Barański M et al.2014).

Meta-analyses demonstrating that the manufacturing technique has a minor impact on the overall phenolics content, such as a 14–26% rise. All of the available meta-analyses point to a somewhat increased phenolic component content in organic food, for certain particular phenolic chemical classifications, significant relative concentration variations of both conventional and organic crops have been documented (Smith-Spangler et al. 2012).

Pesticide residues: In 7% of samples of organic produce and 38% of samples of conventional produce, detectable pesticide residues were discovered. In contrast with conventional produce, organic produce was 30% less prone to contamination with any measurable pesticide residue. By establishing a limit on the maximum quantity of residue from pesticides that may be found on or within any certain food, the Agency of environmental management upholds stringent regulations for the management of pesticides (Lesueur et al. 2007).

Bacterial contamination: Organic produce had a higher risk of contamination, according to four of these five investigations. In sensitivity analysis, when we eliminated the 1 study (of lettuce) that revealed higher contamination among conventional food, we discovered that organic product had a 5% higher chance of contamination than conventional alternatives (Smith-Spangler et al. 2012).

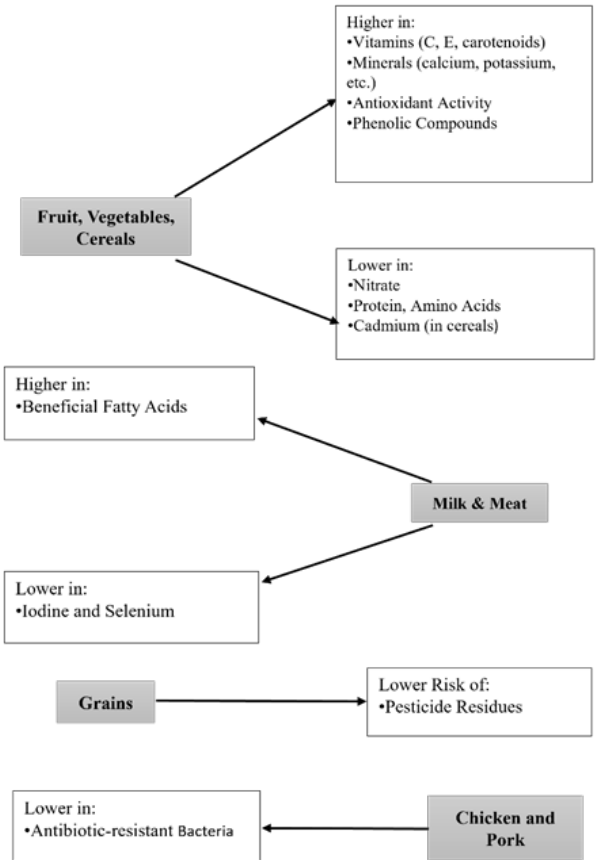

Fig. 1. Organic vs. conventional food composition (Anne Lise et al. 2016).

Consumer perception and attitudes

Taste: There hasn't been any official research conducted to confirm the idea that the texture of organic food is superior

to that of conventional food. There is proof that certain fruit grown organically is drier than fruit farmed commercially (Bourn et al. 2002). Because of the larger concentration of tasting compounds, a fruit that is a little drier might taste stronger. In fact, a lot of chefs and food specialists think that organic foods taste better, giving credibility to the soil care practiced by organic farmers, also substances that plants create to cause their own ripening; as ripening alters the fruit's flavour and texture, this technique might have effects on those characteristics (Axe et al. 2017).

Environmental sustainability: Following years of reliance on chemical inputs and extensive monoculture, agricultural soil will deteriorate to the point that farmers report tougher soils, making farming more challenging and labour-consuming. In most cases, organic farming has lesser of an effect on the environment than conventional farming. Strategies for organic farming can aid in the restoration of species abundance and richness by 30 to 50% as well as the restoration of the ecosystem services that go along with them.

Health and safety

Few scientific studies in humans or animals have investigated whether eating organic food is actually related to better health than eating the corresponding conventional food, Even though numerous studies have analysed the nutrient, antioxidant, and pesticide residue composition of conventional and organic meals (Anne Lise et al. 2016). Specifically, there aren't many long-term clinical trials looking to find possible correlations between health and consuming organic food, mostly because of its high cost. Although herding behaviour also plays an amplifying role, The primary element influencing the consumption of organic food is health consciousness, as seen by the net effect of health awareness being significantly greater than that of herding behaviour (Hurtado et al.2017).

A specific concern with observational studies is that people who frequently purchase organic food typically have healthier eating habits overall and pick more fruits, vegetables, and wholegrain items while consuming less meat (Arvanit et al. 2006). The initial potential trial examining

how weight changes over time regarding the consumption of organic food comprised high purchasers of organic food reported a slower rate of BMI increase over time than low consumers also decrease in the likelihood of obesity was reported. In the Nutrient-Santé study, Organic food consumers, both constant and irregular reported a reduced prevalence of cardiovascular disease however a history of carcinoma, high blood pressure, adult-onset diabetes, and hypercholesterolemia in both genders than nonconsumers (Wang et al. 2023). Many consumers associate food safety with the risk of contamination from disease-causing microbes (such as Salmonella sp. and Coliform) and residues of agrochemicals, primarily pesticides, during the production stage. Conventional and organic farmers both need to follow the same safety standards.

Economic and social benefits

The growing consumer demand for organic products in the industry products is one of the main financial advantages of organic farming. As the number of consumers increases, organic foods are growing and gaining more ecologically conscious popularity and health conscious (Adarsh & Anil. 2022). Preserving the general economic sustainability of farming and ensuring a high enough income to keep farming families in business are two of agricultural policy's top priorities (A. Häring et al. 2001). Another important benefit of organic farming is rural development. Small-scale and family farms, which are essential to preserving the social cohesion and financial stability of rural communities, are typically supported by organic farming. Organic farming supports the creation of robust regional economies that are less dependent on external resources and changes in international markets by promoting sustainable agricultural practices. This fortitude can make the community stronger (Adarsh & Anil. 2022).

Fig. 2. Motives behind purchasing organic goods. (Anubhav & Chidanand. 2022)

CONCLUSION

The recent survey shows that customers are aware of the advantages of the term "organic." Customers congregate in this market not just to purchase food goods but also to brighten and cheer up their days. The findings indicate that people are becoming more conscious of organic food trends and awareness, and farmers are happy to have more clients. Farmers claim that they welcome two to three new families to their market each month.

Future directions

New organic farming practices and technology, including sophisticated pest management and soil fertility increases, can increase yields and reduce sensitivity to environmental pressures.

Conflict of Interest

There is no conflict of interest related to the study.

Author contributions

Acquisition and interpretation of data is done by Arpita Das and Archita Dhara. Conception, design and revising of the article are done by Rupesh Dutta Banik.

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Microbial Fuel Cells: A Sustainable Solution for Wastewater Treatment and Energy Generation

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ABSTRACT

A novel bio electrochemical technology known as microbial fuel cells (MFCs) offers a decentralized, long-term solution to the world's water and energy problems by treating wastewater and producing renewable electricity simultaneously. In this study, we investigate the possibility of MFCs treating wastewater and producing bioelectricity at the same time, highlighting its dual functioning. Through the optimization of electrode materials, microbial consortia, and reactor layouts, this research primarily aims to enhance the performance and efficiency of MFCs. Using carbon-based anodes, proton exchange membranes, and synthetic wastewater, lab-sized MFCs were constructed in this way to create circumstances as close to reality as feasible. Voltage, current density, and power output were used to quantify bioelectricity generation, while microbial activity was used to measure the breakdown of organic contaminants. In addition to consistent power outputs, the results demonstrated a significant decrease in COD, which is indicative of effective wastewater treatment. Energy recovery was greatly improved by increasing the anodic biofilm and electrode surface area, leading to a dramatic increase in electron transfer rates. Additionally, substrate

utilization rates were enhanced through the employment of mixed microbial consortia, which aided in the breakdown of complex organic molecules. According to these findings, MFCs have a lot of promise as a greener and cheaper substitute for conventional wastewater treatment methods. In light of the foregoing, it is reasonable to conclude that MFCs have the makings of a long-term solution for distributed wastewater management and renewable energy generation, particularly in underserved regions. However, there are still significant obstacles to broad adoption, including issues with scalability, operational stability over the long term, and material prices. In order to make this technology more commercially viable and less harmful to the environment, researchers should work on improving MFC designs, maximizing microbial ecosystems, and finding ways to incorporate it into current wastewater treatment systems.

Keywords: *Microbial fuel cells, bio-electrochemical technology, wastewater treatment, renewable energy production, environmental impact*

INTRODUCTION

Nowadays, wastewater is recognized as an essential resource for conserving energy and facilitating water reuse. Conventional aerobic activated sludge and other traditional treatment technologies, conversely, produce residuals and require significant energy input. The challenge they face includes the insufficient recovery of potential resources from wastewater (HE et al 2017). Concerns regarding clean water represent a significant global challenge, exacerbated by factors such as climate change, population growth, and increasing water consumption (Aghababaie et al. 2015). Anaerobic digester (AD) technology is increasingly recognized as a crucial treatment method, given its remarkable effectiveness in converting organic compounds into methane (CH4) gas and its potential for energy savings. This can subsequently be transformed into electrical energy through chemical fuel cells or engines that utilize CH4 as a power source. However, findings indicate that treated wastewater does

not consistently meet stringent regulations, highlighting the necessity for additional technological improvements in the post-treatment phase. In light of these challenges, the practice of water reuse has been extensively implemented, especially in certain arid areas. Nonetheless, the elevated standards for water quality in reuse inherently lead to increased energy demands for treatment (Do et al. 2018). Microbial fuel cells (MFCs) represent innovative bioreactor systems that hold the promise of generating such savings. Nonetheless, discussions continue regarding the future of MFC technology, particularly concerning its commercialization and efficiency. The primary advantage of MFCs lies in their ability to lower energy expenses in wastewater treatment. Logan estimated that if all the energy is recovered as electricity, a population of 100,000 people generating 16.4 × 10^6 (L/year) of wastewater each year could supply enough power for a 2.3 MW power plant through wastewater treatment. Despite the low power density achieved in MFC, utilizing 0.5 MW of power from a wastewater treatment facility is a more practical approach (Aghababaie et al. 2015). This holds particularly true in isolated locations equipped with biosensors, bio hydrogen generators, and local power sources for wastewater treatment and bioremediation (Do et al. 2018; HE et al 2017). This approach facilitates the transfer of electrons to the cathode through electrical flow by oxidizing substrates in an anode chamber with the aid of biocatalyst microorganisms. At the cathode location, reduction processes lead to the formation of water. Microorganisms demonstrate potential as cost-effective substitutes for platinum catalysts, although the latter remains a viable option. Exoelectrogens are microorganisms that function as biocatalysts, possessing unique capabilities such as reducing electron acceptors and facilitating electron transfer to the anode surface. Enhancing MFC technology allows exoelectrogens to be utilized across various applications, particularly in energy generation. The oxidation of organic materials by exoelectrogens generates a redox potential between the electrodes, facilitating the movement of electrons from the anode to the cathode (Jatoi et al. 2021; Enamala et al. 2020; Do et al. 2020). Nonetheless, MFC technology presents a groundbreaking method for improving

energy products and the treatment of waste and wastewater. The mechanisms of electron transfer, the microscale dynamics of biofilm growth and associated transport processes, as well as the macroscale interactions involving electrodes and separators in the bioanode, represent just a fraction of the myriad challenges that require attention (Do et al. 2018; Kim et al. 2015). This article examines MFCs and determines the factors that affect their effectiveness in wastewater treatment and scalable power generation.

MATERIALS AND METHODS

An extensive search was conducted using PubMed, PubMed Central, Google, as well as published research and review articles from various countries. The focus of this review was on the environmental pollutants caused by the leather industries and the microbial bioremediation strategy to eliminate these pollutants. We removed statements of exposure that were too general and only looked at published data. As part of these inclusion requirements, we have included information gathered from credible sources of relevant publications. Other than English, no other languages were considered in the study.

RESULTS AND DISCUSSION

MFCs Configuration

The configuration of a microbial fuel cell Anode and cathode are the two electrodes that make up a microbiological cell, together with a membrane that divides the two compartments. The oxidation of microorganisms at the anode site produces protons and electrons, which initiate the transfer-ring to the cathode. The circuit allows electrons to flow, whereas the membrane allows protons to. When the electrons and protons reach the cathode, they reduce the oxygen to produce water as shown in **Fig. 1**. Mediators facilitate the majority of microbial cells, which are electrochemically inactive. Several operational metrics play a crucial role in evaluating MFC's performance. The current energy landscape clearly indicates a substantial shift towards renewable energy sources is underway. The potential influence of MFC on energy is enormous (Jatoi et al. 2021).

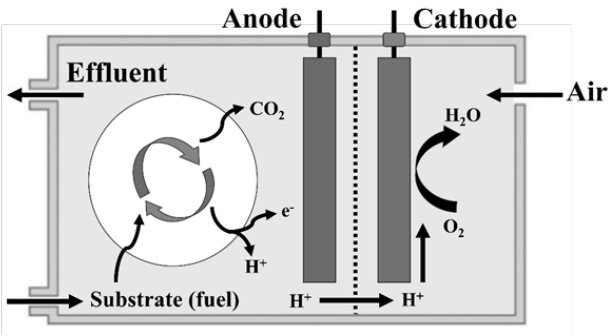

Fig. 1. MFCs Configuration (Schröder 2007)

Elements influencing the efficacy of MFCs

An MFC converts the chemical energy found in organic matter within the substrate into electrical energy. Microorganisms facilitate the oxidation of electron donors such as oxygen, nitrate, or sulphur within the anodic chamber, resulting in the generation of electrons and protons (Jatoi et al. 2021). Countries like the US, Pakistan, China, and India are currently facing an energy crisis, reflecting a global trend. Consequently, technologies rooted in renewable energy sources are crucial for addressing this challenge. The MFC, similar to various other technologies, is shaped by several factors that dictate its performance and efficiency. Multiple parameters can be modified over time, including the cathode, electrolyte, aeration rate, pH, and artificial light, as outlined in Table 1 (Aghababaie et al. 2015).

Table 1. Factors that affect the performance of MFCs (Khan et al. 2019; Jatoi et al. 2018; Jatoi et al. 2020; Rozendal et al. 2006; Choudhary et al. 2017; HE et al 2017; Arun et al. 2020).

Factors	Effects
Cathode	• When scaling up MFC, the cathode determines the maximum power density. • Due to inadequate oxygen transfer crossover, comparatively large amounts of MLC are needed in the lab. • Low cathode liquid Because of the materials used and aeration, a cathode-specific surface area is usually expensive, especially for large-scale MFC. • Power rises from 660 to 1114 MW/m² when carbon paper is replaced with carbon cloth electrodes, a 69% upgrade. The main reason for this gain is a change in cathode material. • Performance is impacted by the cathode's potential since a higher potential cause both more power to be produced and more energy to be lost.
Electrolyte	• Wastewater and distilled water were used in the control experiment; the pH shift was shown to be substantially less than in the non-control experiment. • The temperature of the electrolyte has a significant impact on microbial activity. The ideal temperature varies according to the microbial colony and might range from 20 to 40 degrees Celsius • The pH of the fuel cell's cathode compartment gradually increased to 9.5 following the fuel delivery, whilst the anode's pH dropped. These findings demonstrate the slower rate of proton transfer across the membrane compared to the anode's generation, which the buffer can counteract. • A fuel cell needs a neutral pH to function at its best; hence, buffer is necessary to preserve the ideal microbial environment. • Electrolytes with strong ionic conductivity function better when utilized in MFCs.

Aeration rate	• Ideal aeration increases microbial growth and metabolism it leads to increase in electron transfer and generates power • Regulated aeration leads to an improved current yield. The maximum current increased with an aeration rate of 100 mL min−1, but decreased at 200 mL min−1, likely due to disruption from the sheer force exerted by immobilized microorganisms on the anode. • Control eration minimizes energy loss and regulates oxygen supply • Power generation increased with air flow rate but decreased after it hit a threshold of 150 mL/min. This indicates a reduced capability for MFC generation at air flow rates over the threshold value, most likely due to oxygen blocking microbial anaerobic activity. • It enhances oxygen transport to cathode supporting efficient oxygen reduction reaction.
pH	• An increase of pH in the cathode compartment decreases the current generation; hence, it is beneficial to reduce operational pH for achieving higher power output. • pH ranging from 7 to 8 suppose the increases power density, growth and activity of microbes, it improves electron transfer and generates power. PH exceeding that can reduce electron transfer and decrease power generation • Additionally, the growth of bacteria is crucial for adjusting pH levels. Bacteria typically thrive at a pH level that is near neutral. Variations in pH that facilitate bacterial growth can lead to alterations in other parameters, including ion concentration, proton motility, membrane potential, and biofilm formation. • The enzymatic activity of most prokaryotes is significantly influenced by pH levels. The pH level significantly influences the activity of prokaryotes. • Regular pH monitoring detects fluctuations and adjustments necessary to maintain optimal pH conditions, effectively eliminating acidic or basic components.

Artificial light	• Artificial light, such as light emitting diodes (LEDs), can greatly increase algal growth, which often improves MFC performance. Compared to traditional light sources such compact fluorescent bulbs, the performance and efficiency of MFCs were improved when LEDs were employed as the light source. • Artificial light in waste water accelerates the decomposition of organic contaminants, helping to improve treatment efficiency. • Under blue (0.156-4.741 mW/m2) and red (1.525-12.947 mW/m2) light conditions, power density rises with light intensity (100-900 lux). • Growing algae may cause competition with microorganisms and reduce the efficiency of MFCs. • Using red LED light enhances electron transport from the algal cell to the electrode due to the photosynthetic system's high absorption of light energy at this wavelength.

Electron transfer mechanism in MFCs

The mechanism of electron transfer formed the basis of the operation of the microbial fuel cell (Jatoi et al. 2021). A variety of studies have elucidated the mechanism by which electrons move between microorganisms and the anode/cathode in MFCs. Considering this, direct electron transfer and mediated electron transfer represent two crucial pathways for extracellular electron transfer. The majority of publications indicate that electron transfer in the MET, DET mode takes place between microorganisms and the anode material (Prathiba et al. 2022). Microscopic examinations demonstrated the presence of a significant biofilm layer on the anode of the fuel cell. The generation of power is influenced by various factors, including the kinetic reaction of oxygen, the surface area of the electrodes, electrolytic resistance, and the ability of microorganisms to transfer electrons. Aiyer (2020) examined the impact of operating conditions on a mediator-less microbial fuel cell. The pH must be 7, and the resistance needs to exceed 500 V, based on the conclusions drawn from the study. However, inadequate proton and oxygen supply

was seen when the resistance was less than 200 V; hence, a fuel cell must have a strong reducing activity in order to be effective (Jatoi et al. 2021).

Direct electron transfer (DET)

The bacterial cell membrane or a membrane organelle physically contacts the fuel cell anode to initiate the direct electron transfer. The microorganisms must have membrane-bound electron transport protein relays that move electrons from the inside of the bacterial cell to the outside, ending in an outer-membrane (OM) redox protein that permits the electron transfer to an external, solid electron acceptor (a metal oxide or an MFC anode) (Schröder 2007).

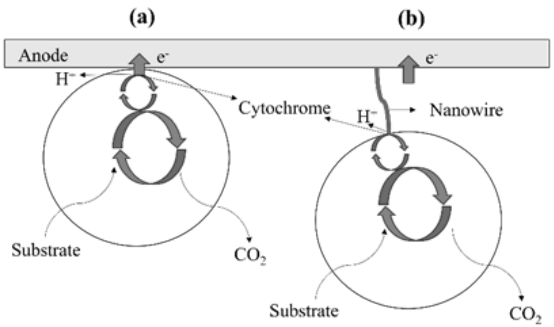

Fig. 2. (a) Membrane-bound cytochrome and (b) Electrically conducting nanowires are used to demonstrate the DET (Schröder 2007).

Mediated electron transfer (MET)

A non-conductive lipid membrane, comprising peptidoglycans and lipopolysaccharides, surrounds various microorganisms, including Saccharomyces species and *E. coli*, which are utilized in some MFCs. This membrane slows down the direct electron transmission to the anode. Hence, the need arises for shuttles or electron mediators which act as carriers for moving the electrons generated in the anodic chamber to

the anode. The ability to pass through the bacterial membrane is necessary for mediators to reach the reductive species within the bacteria and undergo reduction during microbial metabolism. Certain bacteria can use nanowires and pilis, or microbial compounds known as endogenous mediators, to move electrons from the substrate to the anode. We refer to these types of bacteria as electrogenesis bacteria (Aghababaie et al. 2015).

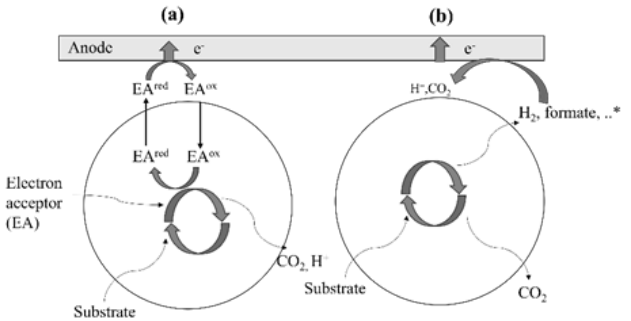

Fig. 3. MET is shown schematically in two simplified ways: (A) through reduced terminal electron acceptors (anaerobic respiration), and (B) by oxidation of reduced fermentation products (Schröder 2007).

Cathodic electron transfer mechanism

There is a relative scarcity of studies that focus on comparing cathodic electron transfer techniques, specifically from cathode material to microbiological cells, with anode electron transfer mechanisms. A study indicates that electrons can transfer from cathode material to the outer cell of bacteria through two distinct pathways: direct and indirect (Kumar et al. 2016). The most prevalent use of cathode electron transfer to microbial cells is for bioremediation of environmental pollutants. Bio-cathodic microbial fuel cells produce methane, other carbon-containing molecules, and hydrogen via the mechanism of electron transfer from the cathode material to

the bacteria (Cheng et al., 2009; Rozendal et al., 2008; Prathiba et al. 2022).

Applications of MFCs

Production of bioelectricity

The chemical energy contained in the chemical components of biomass can be transformed into electrical energy by microorganisms using microbial fuel cells. It has been demonstrated that *R. ferrireducens* can produce energy with an electron yield of up to 80%. The oxidation of formate using Pt black as a catalyst was reported to have an exceptionally high Coulombic efficiency of 97%. For the purpose of sending signals, like temperature, to receivers in remote locations, MFCs are particularly well-suited for powering wireless sensors and compact telemetry systems with low power requirements (Chaudhuri et al. 2003; Do et al. 2018)

Hydrogen bioproduction

It is easy to modify MFCs so that they produce hydrogen rather than electricity. It is possible to store hydrogen for use at a later time. Protons released by the anodic reaction move to the cathode under typical working circumstances, where they mix with oxygen to generate water (Du et al., 2007; Do et al. 2018).

Biosensor

Sensor pollutant analysis, along with in situ process monitoring and control, are further applications for MFC technology. Biosensors for immediate BOD estimate have been constructed utilizing MFC technology, which comprises a transducer with a biological sensing element. The MFC-based sensors have applications for online wastewater monitoring because they are robust over the long term and can potentially use frequently (Feng et al., 2013).

Treatment of wastewater

Studies have demonstrated that biological treatment is a widely utilized, dependable, and very economical method for removing nitrogen and organic debris from leachate. The unique capacity of MFCs employing certain microorganisms to

eliminate sulfides as needed for wastewater treatment. MFCs can confirm that bio electrochemically active microorganisms have strong operational stabilities by promoting their growth during wastewater treatment. There have been reports of a colonic efficiency of up to 80%, and in specific circumstances, up to 90% of the COD can be abolished (Ramadan et al. 2017).

Drawbacks

MFCs present a range of advantages and disadvantages in their field applications. Prior to the commercialization of MFC technology, it is essential to address the operational challenges, such as inadequate power output and elevated running costs. The capital cost of the MFC is generally 30 times higher than that of traditional activated sludge treatment systems for household wastewater, as noted by Rahimnejad et al. in 2014. While MFC technology has seen diverse applications, several challenges remain that need to be addressed for the technology to achieve profitability. Identifying an effective strategy to enhance the MFC represents the initial significant challenge. In evaluating the potential for scaling up previous designs for sustained operations, we identify several constraints, including elevated internal resistance, the distance between electrodes, and the exchange of acolytes and catholytes across the PEM, among others. However, some concepts have been demonstrated but remain unexamined in an industrial context. The second challenge involves the development of cost-effective electrode materials and, if relevant, PEM for MFCs. The electrode materials currently accessible, including carbon paper and carbon cloth, are likely to incur significant costs (Liu et al. 2013).

Activation losses (AL)

Decreases in Activation (AL) Multiple electrochemical processes take place at the electrode surface during the operation of the MFC. An activation energy is necessary for these electrochemical redox processes. This activation energy is essential for facilitating electron transport to the electrolyte/feed oxidation at the anode or for the reduction of O2 as a terminal acceptor at the cathode. Increasing the process temperature to a specific level may lead to a reduction in the

AL. In a similar manner, it can be minimized by improving electrode catalysis through the incorporation of a catalyst into the electrode material. Conversely, the development of biofilm on the electrode surface, along with the expansion of the electrode surface area, can lead to suboptimal AL (Do et al. 2018).

Concentration polarization (CP)

When the electrode side oxidizes the substrate more quickly than it could be transported towards the electrode (anode) surface, CP occurs. The greater oxidative strength of the anode in MFCs is generally thought to be the cause of this. This phenomenon might be troublesome, though, in situations when flow is significantly slowed down, such as in dense, non-conductive anodophilic bacterial biofilms, hydrodynamics, and geometrical characteristics (Do et al. 2018).

Ohmic losses (OL)

Interconnections, resistance to ion transfer via membranes, and resistance to ion transfer through electrolyte were the primary causes of OL in MFC. But, because of poor connections or decreased electrolyte conductivity, the resistance across the MFC may increase quickly. The electrolyte's free stream, bacterial biofilm growth, high conductivity, and adequate turbulence for adequate H^+ flow towards the separator and cathode electrode materials must all be maintained by the anode material arrangement. To reduce cathode resistance (CR), active exoelectrogenic microorganisms or materials can be used as a biocatalyst or catalyst. Increases in the cathode's specific surface area and mass transport rate may also result in a reduction in the CR (Do et al. 2018).

Potential losses (PL)

The thermodynamically predicted potential, or cell electromotive force (Ethermo), is always higher than the operating voltage (Vop) produced by MFC. There is no way to reverse these possible losses. Energy loss in MFCs can happen in a number of ways, including activation loss (perhaps for starting the reactions on both electrodes and extracellular electron transfer to the anode), bacterial metabolism loss

(because bacteria get energy by oxidizing the substrate), mass transfer loss (because of the reactants' restricted flow to the electrode), and ohmic losses (because of charge transfer resistance and proton diffusion resistance) (Du er al. 2007)

CONCLUSION

Wastewater is acknowledged as a significant factor in environmental pollution. The current energy and economic constraints of modern wastewater treatment technology make it challenging to achieve and maintain wastewater recovery. Microbial fuel cells (MFCs) have undergone extensive investigation and are now acknowledged as a groundbreaking technology, especially notable for their benefits in the wastewater treatment field. This study outlined the essential architecture and design characteristics of microbial fuel cells. A notable characteristic that sets MFCs apart from other wastewater treatment techniques is anaerobic digestion (AD). MFCs improve energy generation, reduce sludge buildup, and promote energy conservation. The main MFCs used for wastewater treatment were contaminated with anaerobic or aerobic sludge, thereby eliminating the need for a mediator. Optimal pH and temperature, akin to other microbial systems, promote bacterial proliferation and thereby increase MFC effectiveness. Salinity and ionic strength represent additional essential factors. Elevated salinity and ionic strength can improve the conductivity of the substrate and boost MFC performance, despite the negative impacts of salt on microbial growth. The composition and design of the anode, cathode, and membrane play a crucial role in determining the cost and efficiency of MFCs and should be carefully evaluated throughout their development process.

FUTURE SCOPE

MFCs demonstrate effectiveness in addressing hazardous wastewaters, particularly those from industrial sources that are laden with heavy metals and toxic compounds. Saline wastewaters can be processed in this way, depending on their compatibility with the salinity levels of the anode chamber. As a result, MFC could be considered promising systems for addressing various forms of wastewater treatment.

The electricity generation from MFC is minimal; however, wastewater treatment remains a primary focus in this area. Taking into account the previously discussed factors is essential for attaining the best outcomes in wastewater treatment and energy production. Metabolic engineering can be utilized to enhance bacterial metabolism, thereby boosting cell potential when energy generation is desired. The combination of electricigenic bacteria with fundamental substrates such as acetate, considering the design factors, could yield a higher potential. In wastewater treatment processes, the use of modified bacteria is impractical; consequently, activated sludge is often used as the inoculum for microbial fuel cells (MFC). The expansion of MFC is a debated issue, and there is a lack of comprehension surrounding it. The creation of innovative materials and technologies for MFC production, coupled with an examination of the elements affecting MFC performance, will enhance the implementation of MFCs in extensive wastewater treatment processes. In the construction of an MFC, it is essential to take into account the material and surface area of the anode, cathode, and membrane, along with the distances between electrodes and the potential for scalability. In summary, developing a cost-efficient MFC for scalable wastewater treatment that also boasts significant power generation potential is beneficial.

Conflict of Interest

There is no conflict of interest related to the study.

Author contributions

Acquisition and interpretation of data is done by Arijit Banerjee, Kalyani Shaoo, Khondekar Maksuda Begam, Katyayini Singh, Amima Maswood and Aritri Josh. Conception, design and revising of the article are done by Rupesh Dutta Banik.

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Engineering heart health: Biotechnology's innovation in cardiovascular disease management

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ABSTRACT

Cardiovascular diseases (CVDs) are a leading cause of death worldwide, presenting a significant challenge to healthcare systems. Recent advancements in biotechnology are transforming the management of CVDs, offering innovative solutions for diagnosis, treatment, and prevention. This review explores key biotechnological innovations, including gene therapy, regenerative medicine, precision medicine, and advanced diagnostics. Gene-editing technologies like CRISPR-Cas9 are promising in treating genetic cardiovascular conditions, while stem cell therapy and tissue engineering enable heart tissue repair. Precision medicine allows for more targeted treatments, and new diagnostic tools, enhanced by artificial intelligence, are improving early detection and risk assessment. Bioengineering has also led to the development of advanced cardiovascular devices, such as biocompatible stents and artificial hearts. Despite these advancements, challenges such as high costs, technical limitations, and ethical concerns remain. The integration of nanotechnology, 3D printing, and digital health tools could further revolutionize cardiovascular care. In conclusion, biotechnology holds immense potential to improve CVD management, reduce healthcare burdens, and enhance patient outcomes through personalized, innovative therapies.

Keywords: *cardiovascular diseases, CRISPR-Cas9, tissue engineering, artificial intelligence, nanotechnology*

INTRODUCTION:

Cardiovascular diseases (CVDs) refer to a variety of heart and blood vessel illnesses. Diseases of the heart muscle and the circulatory system that supply the heart, brain, and other essential organs are among the many conditions that fall under the broad category of cardiovascular disease (Pagidipati et al. 2013). They are a group of diverse illnesses, with atherosclerosis serving as the most common underlying cause. CVDs are long-term, asymptomatic conditions that progressively worsen throughout a person's life. Furthermore, the primary cause of illness and mortality among patients globally is CVDs (Sonja et al. 2014). Globally, atherosclerosis is the leading cause of death from cardiovascular disease. It is a thickening and hardening of the artery wall that occurs with aging and is linked to several disorders and has a significant negative influence on the cardiovascular system. The development of atherosclerosis is largely caused by elevated plasma cholesterol levels (>150 mg/dL) (Helvaci et al. 2019; Frak et al. 2022).

Globally, the prevalence of CVDs, the primary cause of premature death and disability in humans, is increasing. In addition to contributing significantly to the rising expenses of healthcare, CVDs have a significant socioeconomic impact on the general populace. Nearly all cardiovascular diseases have an underlying pathogenesis and progression that is primarily of atherosclerotic origin. This causes peripheral vascular disease, coronary artery disease, cerebrovascular disease, and venous thromboembolism, which in turn causes myocardial infarction, cardiac arrhythmias, or stroke. Lipid-lowering medications, antihypertensives, antiplatelet, and anticoagulant treatments are the main regimens for the prevention and treatment of CVDs, in addition to adopting healthy lifestyle choices. There are still large gaps in the treatment of CVDs, notwithstanding their efficacy (Flora et al. 2019).

The biggest cause of death, accounting for 17.7 million fatalities, is cardiovascular disease (CVD), which includes ischemic heart disease and cerebrovascular diseases like stroke. The World Health Organization claims that India is responsible for one-fifth of these deaths globally, particularly among younger people. India has an age-standardized CVD death rate of 272 per 100,000 people, which is significantly higher than the global average of 235, according to the Global Burden of Disease research. (Prabhakaran et al. 2016) Indians are afflicted with CVDs ten years before people in the West. The high mortality rate, quick progression, and early age of beginning of CVD are of great concern to us Indians. Indians are known to have the highest rates of coronary artery disease (CAD), and this elevated risk cannot be explained by the traditional risk factors (Sreeniwas et al. 2020).

Cutting-edge biological therapies with the ability to repair and regenerate damaged cardiovascular tissues include gene therapy, stem cell therapy, and RNA-based therapeutics. Early findings are encouraging, indicating that these treatments could significantly alter the landscape of CVD treatment, even if they are still in different phases of clinical validation. The care of CVD is being revolutionized by cutting-edge medical technologies and gadgets, including wearable technology, minimally invasive procedures, and implantable devices (Di et al. 2024). By moving care from hospitals to homes, these developments improve patient outcomes and lower healthcare costs by enabling early diagnosis, ongoing monitoring, and efficient treatment. Thanks to genetic testing and biomarker identification, personalized medicine enables customized treatments that maximize therapeutic benefits and reduce side effects (Roth et al. 2020). However, there are a number of obstacles to the widespread use of these new treatments, such as ethical concerns, cost and accessibility concerns, and regulatory barriers. Accelerating the creation and application of novel treatments requires addressing these obstacles and encouraging interdisciplinary cooperation. The management of cardiovascular disease could be revolutionized by incorporating new therapeutic approaches into cardiovascular care (Singh et al. 2024).

Biotech advancements in cardiovascular diagnosis:

Recent biotechnological advancements have significantly enhanced cardiovascular diagnosis, making it more precise and non-invasive. Innovations such as genetic testing, biomarker discovery, and advanced imaging techniques like 3D echocardiography and cardiac MRI enable earlier and more accurate detection of cardiovascular diseases. Furthermore, artificial intelligence (AI) is playing a key role in interpreting complex data from diagnostic tools, aiding in the prediction of heart disease risks. These technologies are improving the ability to diagnose conditions like heart failure, arrhythmias, and coronary artery disease, thus allowing for better-targeted treatments and improved patient outcomes (Tique et al. 2024).

Nanotechnology: The therapy of cardiovascular illnesses, especially atherosclerosis and myocardial infarction, has shown great promise in the realm of nanotechnology. Targeted drug distribution made possible by nanosystems is a crucial feature that increases treatment efficacy and reduces adverse effects (Bonnet et al. 2021). Furthermore, by creating molecular imaging agents that selectively target atherosclerotic plaques, nanosystems aid in diagnostic imaging and make it possible to identify and diagnose severely sick locations (Mog et al. 2019).

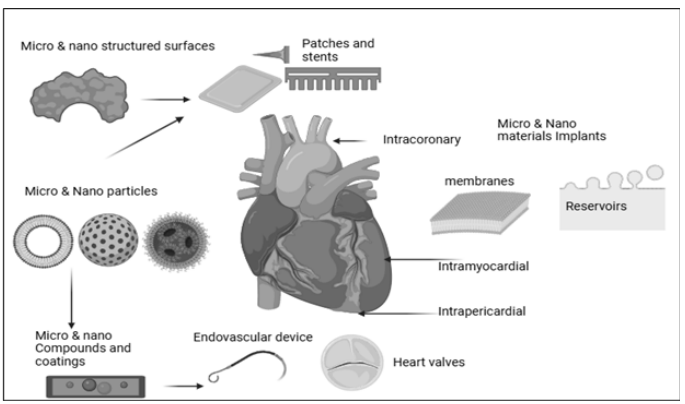

Fig. 1. Nanotechnology in cardiovascular dieseases (Omidian et al. 2023).

Nano-based systems in Atherosclerosis: Bonnet et al. (2021) presented a study on nanosystem that made use of nano-emulsions of oil in water that were loaded with nanoparticles of superparamagnetic iron oxide. A human antibody (P3) functionalizes this nanosystem, allowing for targeted magnetic resonance imaging (MRI) of atheromatous lesions that pose a high risk. Additionally, it can deliver medications to atheromatous plaques, opening the door for non-invasive atherosclerosis imaging and treatment approaches.

Hu et al. (2022) investigated a unique nanosystem using PdH nanozymes that resembled tetrapod needles. Through ROS scavenging, hydrogen-based anti-inflammatory actions, and autophagy activation, these multifunctional nanozymes efficiently reduce inflammation and oxidative stress in arterial plaques. The study emphasizes the potential of developed nanomedicines in the treatment and management of atherosclerosis.

The development of lipid-core nanocapsules with anti-PECAM-1 on their surface and docosahexaenoic acid (DHA) as its nucleus allowed for targeted distribution to the inflammatory endothelium in atherosclerosis. Without compromising cell viability, the nanocapsules showed effective conjugation, an appropriate size, and cellular uptake. These results point to the created nanocapsules' potential for use in pharmaceutical treatments for atherosclerosis (De Castro et al. 2021).

Systems of biomimetic nanoparticles have been studied for the targeted treatment of atherosclerosis. Rapamycin-loaded PLGA nanoparticles coated with a macrophage membrane exhibit biocompatibility and specific binding to atherosclerotic lesions and activated endothelial cells. The nanoparticle technology demonstrated good safety profiles and prevented the advancement of atherosclerosis in animal models. For the treatment of atherosclerosis, biomimetic nanoparticles may be used as secure and efficient drug delivery vehicles (Wang et al. 2021).

PM@Se/Rb1 NPs, a biomimetic nanodrug delivery system, was created to specifically treat atherosclerosis. To improve plaque

stability, the method used ginsenoside Rb1 nanoparticles (Se/Rb1 NPs) and platelet membrane coating on selenium (Se). When paired with the anticoagulant medication warfarin, the PM@Se/Rb1 NPs had anti-inflammatory and anti-angiogenic properties, efficiently transported medications to plaques, and shown possible synergistic effects. This study demonstrates the potential of biomimetic nanodrugs and how they can be used in conjunction with clinical medications to treat atherosclerosis (Yin et al. 2022).

Gene therapy: In cardiovascular medicine, gene therapy has become a game-changing field that offers novel ways to treat complicated diseases like CVD. In order to modify gene expression and enable targeted therapeutic treatments, a variety of molecular tools have been created in this field. With the use of these advanced instruments, scientists can target particular genes, alter gene expression, and take molecular action, providing potential methods to lessen the underlying genetic causes of CVD (Cring et al. 2022).

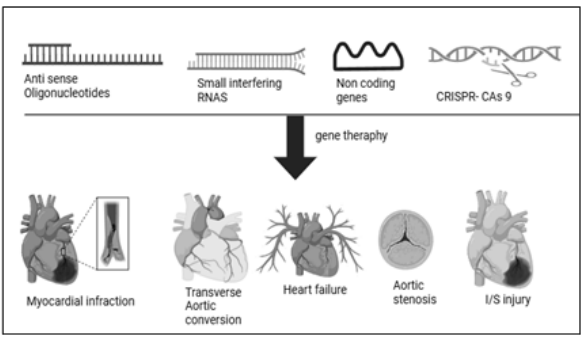

Fig. 2. Gene therapy for cardiovascular diseases (Crooke et al. 2017).

Antisense oligonucleotide: ASOs function to suppress the expression of a particular molecular target. They are composed of single-stranded DNA and usually contain 13–20 nucleic acids. This is accomplished by attaching itself to mRNA and creating a duplex that RNAse H1 cleaves, which prevents the creation of proteins (Crooke et al. 2017).

Crucial cardiovascular trials have shown great promise for RNA-targeted ASOs, especially those that target Apolipoprotein C-III (ApoC-III) (Ramms et al. 2019). Triglyceride-rich lipoproteins are cleared more slowly when ApoC-III inhibits lipoprotein lipase (LPL), which raises the risk of cardiovascular disease. The first generation of volanesorsen, an ASO that targets ApoC-III, has been shown in clinical trials to be effective in lowering TG levels by up to 77% and 71.8%, respectively (Berthold et al. 2021). This suggests that individuals with hypertriglyceridemia may benefit from using volanesorsen. By efficiently lowering TG levels by 78%, GalNAc-volanesorsen, which is licensed in the EU for FCS, reduces the risk of acute pancreatitis. GalNAc-conjugated volanesorsen targeting ApoC-III has been investigated in clinical trials, with encouraging results in lowering atherogenic lipid profiles and enhancing safety profiles (Alexander et al. 2019).

A protein that is mostly produced in the liver, angiopoietin-like 3 (ANGPTL3) is a unique genetic target for lowering the risk of CVD since it is essential in blocking endothelial lipase and LPL (Li et al. 2020). The lipid profile of individuals with familial mixed hypolipidemia was effectively recreated by a GalNAc-modified ASO in phase 1 research, suggesting the possibility of therapeutic mimicry. ANGPTL3 suppression significantly reduced ApoB, ApoC-III, non-HDL-C, and VLDL-C. Inhibition of ANGPTL3 was investigated for clinical safety in a phase 2 experiment (NCT03371355) (Minicocci et al. 2013). Multiple doses resulted in a maximum 63% reduction in TGs and a 36.6% reduction in non-HDL-C levels, according to a phase 1 study conducted in humans (Akaumianakis et al. 2021).

Small interfering RNA(siRNA): The potential of chemically produced or recombinant siRNAs to initiate RNA interference and use the RNA-induced silencing complex (RISC) molecular machinery was investigated. From the first lipid-mediated transfection reagents to more sophisticated formulations, the administration of these treatments has changed throughout time (Ranasinghe et al. 2023).

Over the last ten years, a number of pharmacological treatments for ATTR amyloidosis have been developed. In August 2018, the FDA approved ONPATTRO (formerly Patisiran), the first marketed siRNA (Kristen et al. 2019). Unlike its rivals that are GalNAc-conjugated, ONPATTRO has a different liver uptake mechanism. ONPATTRO, which is formulated as lipid NPs (LNPs), protects therapeutic oligonucleotides from being broken down by natural enzymes. The particle enters the liver by vascular fenestrations and attaches itself to LDLR on hepatocytes after being opsonized by apolipoprotein E (ApoE), which is produced from circulating lipoproteins (Akinc et al. 2019). ONPATTRO showed a safe and efficient decrease in serum TTR in phase 1 and phase 2 trials. Patients with ATTR-polyneuropathy were the subject of the phase 3 APOLLO study, which showed promising results for 126 patients with related cardiac dysfunction (Di Stefano et al. 2022).

The liver produces the majority of angiotensinogen (AGT), which has drawn interest as a possible target for cutting-edge antihypertensive treatments. In pre-clinical research and a phase 1 study, zilebesiran—the first siRNA created to lower AGT mRNA in hepatocytes showed effects exclusive to the liver (Matsusaka et al. 2012). Notably, four-part phase 1 research found that zilebesiran single doses resulted in clinically significant drops in both systolic and diastolic blood pressure that lasted for up to 24 weeks. The drug's effectiveness was demonstrated when the serum AGT decrease surpassed 90% (Desai et al. 2023).

Biomarkers: A biomarker is a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (Downing et al. 2001). Both the assessment of sickness and the creation of medication therapy for illnesses depend heavily on biomarkers. Biomarkers can even be useful in figuring out the precise dosages for any particular medicine in the latter stages of drug development. Biomarkers are also being regarded as surrogate end goals for therapeutic studies in more recent times (Dhingra et al. 2017).

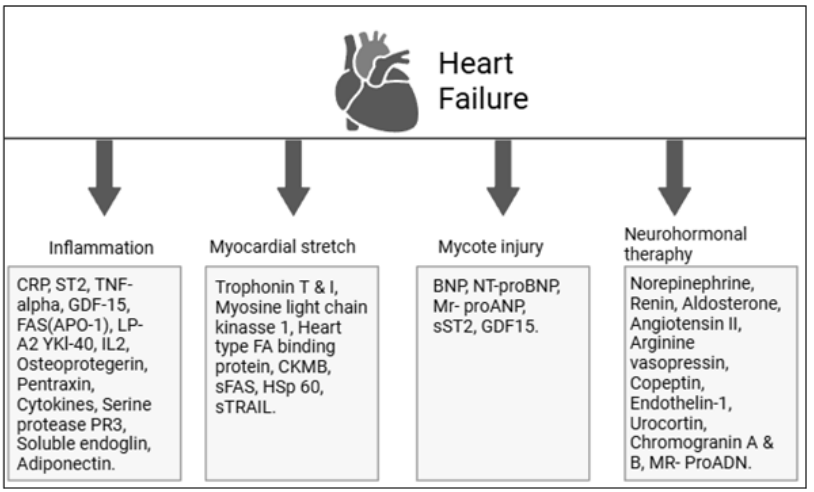

Fig. 3. Biomarkers for heart failure (Dhingra et al. 2017)

The following are the biomarkers used for heart failure:

Troponin I and T: They are cardiac isomers of the troponin-tropomyosin complex proteins, are released into the bloodstream by cardiomyocyte necrosis and are often helpful in identifying myocardial ischemia. Nevertheless, high-sensitivity Troponin T and I tests have been suitably investigated for the prognostication of patients with established heart failure and for the prediction of heart failure as they are also enhanced in the blood of patients with severe heart failure (Saunders et al. 2011; Xue et al. 2011).

Neutrophil gelatinase-associated lipocalin (NGAL): Renal tubular cells release neutrophil gelatinase-associated lipocalin (NGAL), another glycoprotein covalently bound to matrix metaloproteinase-9, in response to renal inflammation and injury. In the GALLANT trial, NGAL was also demonstrated to provide additional prognostic and diagnostic value in addition to BNP (Maisel et al. 2011).

Galectin-3: An intriguing biomarker, galectin-3 plays a crucial part in the initiation and control of cardiac remodelling and

fibrosis. On short-term follow-up, the blood Galectin-3 level in patients with acute decompensated heart failure has been demonstrated to be predictive of mortality. However, there is less evidence in the literature to support the independent use of Galectin-3 alone to predict prognosis in patients with heart failure (Van et al. 2006; Shah et al. 2010).

ST-2: The interleukin family (IL-33) receptor ST-2 has two gene forms: soluble (sST2) and transmembrane. Blood ST-2 levels have been demonstrated to predict mortality and new-onset heart failure, just as other biomarkers (Sanada et al. 2007). In patients with ST-elevation myocardial infarction, researchers have also looked at the predictive power of ST-2 levels in addition to other conventional risk markers including NT-proBNP levels. Additionally, it influences prognosis more so than conventional heart failure risk factors (Sabatine et al. 2008).

Mid-regional pro-adernomedullin (MR-proADM): The existence of chronic heart failure is closely linked to higher levels of mid-regional pro-adernomedullin (MR-proADM), a stable prohormone fragment of adernomedullin, a vasodilatory peptide. It has been demonstrated that MR-proADM is more accurate than BNP and NT-proBNP at forecasting 90-day death in patients with heart failure and dyspnoea (Maisel et al. 2010).

Growth differentiation factor-15: Researchers have connected elevated levels of this biomarker, which is categorized as having anti-hypertrophic effects (apoptosis), to evaluate prognosis in patients with chronic heart failure and in community-dwelling adults as a predictor of all-cause mortality, including non-cardiovascular mortality, in addition to the data provided by blood NT-proBNP and C-reactive protein levels (Daniels et al. 2011).

Precision medicine: Through the use of "omics" in cardiology, precision medicine has the potential to completely transform the treatment of cardiovascular disease (CVD) in the future. It allows a doctor to treat heart conditions according to each patient's particular profile. Here are the examples of Omic stratified literature on heart failure: Improved spironolactone

responsiveness in diastolic heart failure patients was associated with NR3C2, which codes for the spironolactone target protein, and CYP11B2, which is involved in aldosterone production. In individuals with transthyretin-associated cardiomyopathy, Tafamidis decreased cardiovascular event-related deaths and hospitalizations (Maurer et al. 2018). BAG3 and CDKN1A are linked to LV systolic dysfunction, while loci KLHL3 and SYNPOL2-AGAP5 are implicated in HF. Numerous processes that contribute to the evolution of heart failure are impacted by spiro lactone. Patients with heart failure can have their 5-year all-cause mortality predicted by copeptin (Glick et al. 2013). A CA125-guided diuretic approach increased eGFR in patients with acute heart failure and renal dysfunction, and CA125 is a proxy of fluid overload, making it potentially useful for directing decongestion treatment. Those with AHF have high levels of hs-cTnT. Whether at baseline or peak, a rise in hs-cTnT is linked to adverse outcomes, particularly cardiovascular mortality at 180 days. The long-term trajectory of cardiac troponin T (cTnT) and N-terminal pro-brain natriuretic peptide (NT-proBNP) in older persons without HF can predict systolic dysfunction, incident HF, and CV mortality (Sethi et al. 2023).

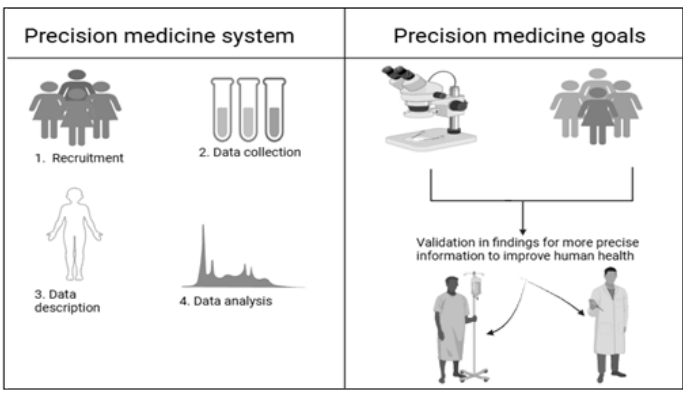

Fig. 4. Precision medicine (Bamba et al. 2024).

Cardioinformatics: Cardioinformatics is an interdisciplinary area that uses computational biology and bioinformatics techniques to store, compute, and analyze data related to

cardio- (vascular, renal, and metabolic) diseases. In order to meet clinically unmet requirements, cardioinformatics techniques can assist medical practitioners in comprehending the connections between cardiovascular health and other physiological systems. They can also offer insights into how to diagnose, treat, and manage these complicated disorders more efficiently. In order to gain new insights into the underlying causes of cardio- (vascular/renal/metabolic) diseases and to increase the accuracy of diagnosis and treatment in the clinic and at home (e.g., using wearable devices like continuous glucose or blood pressure monitors), cardioinformatics entails the collection, management, and analysis of large amounts of human and animal data (Khomtchauk et al. 2020).

Machine learning, artificial intelligence (AI), and other cutting-edge computational methods have frequently been used in cardioinformatics research to evaluate intricate biological data and create predictive models that can guide clinical judgment in real-world applications ranging from the identification of new drug targets to medical imaging (such as cardiac electrophysiology). To find effective, existing medications that could help patients with cardiovascular diseases or their related renal and metabolic co-morbidities (e.g., chronic kidney disease, type II diabetes, non-alcoholic fatty liver disease, etc.), for example, it has been common practice to use AI and machine learning techniques to search genome sequencing results and large drug-target databases (Tsao et al. 2023).

Similarly, in the context of routine medical practice and precision medicine, advances in cardioinformatics can help doctors and healthcare providers interpret different kinds of patient-centric data, such as genetic, proteomic, metabolomic, and imaging datasets (e.g., MRI, CT scans, echos). When combined, cardioinformatics aims to increase our quantitative knowledge of the cellular and molecular processes that underlie the pathophysiology of cardio-(vascular/renal/metabolic) diseases and to create more efficient data-driven approaches to their diagnosis, treatment, and prevention, which will ultimately improve patient outcomes (Khomtchauk et al. 2023).

Cardiovascular MedTech: The increasing availability of a wide variety of linked health devices holds great promise for revolutionizing CVD preventive and management tactics to enhance health outcomes and lessen the financial burden of CVD. In addition to prevention, disease management, patient involvement, self-care, and education, linked health technology for CVD also aims to enhance physicians' capacity to offer patient care through remote monitoring techniques and tools. Providing safe, efficient, individualized care at every stage prevention, early detection, diagnosis, treatment, and management is one of the main goals of this developing discipline (Wongvibulsin et al. 2019).

Cardiovascular MedTech advancements include minimally invasive treatments in addition to diagnostics. Smaller, more accurate tools have been made possible by the development of new materials and robots, which has sped up recuperation periods and allowed for less intrusive procedures. The creation of patient-specific implants and prostheses has been transformed by the use of 3D printing technology, providing customized solutions for challenging situations (Vento et al. 2024).

Wearable technologies: The capacity to continually measure and communicate important physiological data in real time is unparalleled thanks to wearable technology that is outfitted with advanced sensors. Utilizing wearable technology allows medical practitioners to get a thorough picture of the patient's vital signs, such as blood pressure, heart rate, and oxygen saturation, which promotes a more sophisticated and individualized approach to cardiovascular treatments (Guk et al. 2019). The Nexfin® makes use of "Nexfin HD," a non-invasive monitoring device that uses continuous pulse pressure to give precise and ongoing cardiovascular system evaluation (Ariturk et al. 2016). By employing a sophisticated oscillometric approach to identify changes in blood pressure, the Continuous Noninvasive Arterial Pressure (CNAP®) provides continuous and noninvasive arterial pressure measurement (Biais et al. 2010).

Robotic technologies: Cardiovascular procedures are now much more capable thanks to robotic technology, which

provide medical personnel more accuracy and dexterity. Procedures may be carried out more precisely by utilizing robotic help, enabling complex movements that were previously difficult with conventional methods (Chakravartti et al. 2019). The da Vinci Surgical System has transformed procedures including mitral valve repair and coronary artery bypass grafting (CABG). The overall effectiveness of these surgeries is increased by the system's robotic arms, which are operated by a surgeon and offer unmatched precision in navigating confined locations (Cerny et al. 2020). The incorporation of robotic technology into catheter-based therapies is best demonstrated by the Hansen Sensei Robotic Catheter System. By enabling very precise and controlled motions during operations such as catheter ablation for arrhythmias, this technology lowers the margin of error and enhances patient outcomes (Nazafi et al. 2023).

3D Printing technology: A revolutionary development in personalized medicine and therapeutic treatments is the use of 3D printing technology in the cardiovascular profession. This cutting-edge technology makes it possible to precisely fabricate anatomical models that are unique to each patient, providing physicians with essential resources for preoperative planning, medical education, and patient communication. For instance, bioprinting has not yet been widely applied in the field of cardiovascular medicine for a number of reasons. Creating sophisticated, hierarchical, and biocompatible artificial tissue constructions is currently the main application for the technique. Currently, training and preintervention planning are the primary uses of 3D printing in the cardiovascular area. Even while 3D printing makes it possible to create insightful anatomical models, it's important to keep in mind that most existing models are static and might not accurately capture the dynamic nature of either normal or diseased tissues (Bartel et al. 2018; Liu et al. 2021).

Novel materials: Cardiovascular devices with improved durability and customized biomechanical qualities may now be designed thanks to the discovery and integration of innovative materials including shape memory alloys and biocompatible polymers. For stents, grafts, and other

implanted devices to function as best they can and be biocompatible, certain materials are essential (Song et al. 2022). Advanced biocompatible polymers were used to create artificial heart valves, which are more adaptable and wear-resistant than traditional devices. These features lower the chance of deterioration and enhance the quality of life for individuals with cardiac valve disorders by enabling the valves to operate more effectively over time (Kumar et al. 2023). In order to meet the increasing need for appropriate replacements in patients with cardiovascular illnesses, vascular grafts and endovascular procedures composed of biocompatible and long-lasting materials are crucial (Fang et al. 2021).

Challenges and limitations: Even though nanosystems have a lot to offer in the areas of targeted drug delivery, cardiovascular disease treatment, and diagnostics, these issues must be properly taken into account and resolved for their effective clinical use. Shear-targeted drug delivery, magnetic delivery devices, and liposomes all exhibit promise, but further study is needed to maximize these strategies and guarantee the effective delivery of medications to the central nervous system. Improving penetration through the blood–brain barrier and overcoming microvascular dysfunction are two persistent issues that must be resolved to increase the effectiveness of nanosystems in the treatment of stroke (Omidian et al. 2023).

Low editing efficiency, a clear off-target effect, high toxicity and low tissue specificity of the delivery system, inadequate identification of the pathogenic genes of cardiovascular disease, and other flaws prevent gene editing therapy from being used clinically at this time (Cao et al. 2021). Despite providing real-time monitoring capabilities, wearable technology may face issues with data quality, dependability, and user adherence that might reduce its usefulness in therapeutic settings. To ensure dependability and safety, artificial intelligence must be thoroughly validated and seamlessly integrated into current healthcare operations, even while it holds promise for precise and customized therapies. Although robotic technology and minimally invasive techniques improve surgical precision

and lessen physical stress, they may also pose challenges for healthcare practitioners in terms of cost, accessibility, and the learning curve (Wongvibulsin et al. 2019).

To guarantee sustainability in healthcare delivery, it is critical to take into account the ecological and financial effects of these technologies. Furthermore, it is impossible to overestimate the importance of carrying out a large number of clinical studies in order to validate the safety and effectiveness of the suggested medical technology. These studies are necessary to guarantee the positive effects of these innovations on patient health and well-being and to provide evidence-based support for their broad adoption (Maddula et al. 2022).

CONCLUSION:

In conclusion, advancements in biotechnology are significantly transforming the landscape of cardiovascular disease (CVD) prevention, diagnosis, and treatment. Innovations in areas such as genetic engineering, tissue engineering, nanotechnology, and biomaterials are enabling more effective, personalized, and minimally invasive approaches to heart health. Promising developments, including targeted drug delivery systems, gene therapies, and bioengineered cardiac tissues, offer the potential to address the underlying causes of cardiovascular conditions and improve patient outcomes. Despite these advancements, challenges such as regulatory barriers, high costs, and the need for further clinical validation remain. Ongoing collaboration across disciplines—including engineering, biology, medicine, and regulatory agencies—is critical to maximizing the impact of these technologies. As research progresses, biotechnology has the potential to significantly enhance the management and treatment of cardiovascular diseases, offering hope for better heart health worldwide.

FUTURE PROSPECTIVES:

Unquestionably, genomics has sped up the process of identifying the mutations that cause heart disorders. Although the technology required to convert this data into clinical interpretation and practice is still difficult, the exploration of genetic sequences, gene assembly, and identification is still

developing and appears to have a bright future. The exome, or protein-coding DNA, is the subject of much research, but non-protein-coding DNA and its influence on significant clinical illnesses, which are mainly unknown, are also of interest in modern genetic sequencing. Given the chronic, multimorbid, and diverse character of cardiovascular illnesses and their slow development, precision medicine is the way of the future and has the potential to improve their care. These illnesses may start their etiology decades before they finally show symptoms. Because cardiovascular illnesses may be prevented, diagnosed early, and treated specifically, the application of precisely focused diagnostic and individualized therapy techniques can completely change management. Many of the technology required for the application of precision medicine are still in their infancy, and the discipline is still developing. Traditional cardiology has benefited greatly from these new methods in many areas, such as the pathophysiology, diagnosis, and treatment of diseases. The pathophysiology of cardiovascular disease has been better understood because to gene analysis and transgenic methods. Despite the current lack of clinical success, gene therapy and stem cell therapy have given scientists and doctors new ideas for treating cardiac disorders. Consequently, there is a pressing need for substantial and quick advancements in molecular cardiology. The creation of novel technologies in molecular and cellular biology is primarily responsible for these advancements.

Conflict of Interest

There is no conflict of interest related to the study.

Author contributions

Acquisition and interpretation of data is done by Dipti Das, Tanisha Bhowmick, Tiyasha saha. Conception, design and revising of the article are done by Rupesh Dutta Banik.

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Significance of gut microbiota in human health and illness

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ABSTRACT

The immune system and commensal microbiota are constantly interacting, with the former teaching the latter how to react to antigens. The human gut is home to a diverse range of microorganisms that are now understood to be important for the human host by controlling immunological homeostasis and metabolic processes. Manipulation of the intestinal microbiota has been shown to change host immune cell homeostasis in mouse studies. Furthermore, metagenomic-sequencing analysis has shown that patients with obesity, asthma, and inflammatory bowel disease have different gut flora. A weakened immune response and decreased tolerance to commensal microbes are the outcomes of disruptions in the composition of the microbiota. A number of tactics, including probiotic medication, nutrition, and antibiotic use, have been used to prevent or treat chronic inflammatory disorders because of the changed microbiota composition linked to certain inflammatory diseases. Presenting and discussing current data demonstrating that the gut microbiota regulates immune system activity is the aim of this study.

Keywords: *Microbiota, homeostasis, metagenomic sequencing, gut flora, chronic disorders.*

INTRODUCTION

In an environmental niche, the collective genomes of microorganisms are referred to as the "microbiome," while the microorganisms themselves are known as the "microbiota" (Sarkar et al. 2021; Pallister et al. 2016). Beginning in early childhood, the signature core microbiome is established and linked to early life events and maternal pregnancy-related factors, including delivery type, formula feeding, gestational age (GA), adverse childhood experiences, antibiotic exposure, and ecological factors (Sarkar et al. 2021). The human microbiome is essential to human health because, depending on its precise makeup, it can have trophic, protective, and metabolic roles (Laing et al. 2018). The human gut's microbiota ecosystem depends heavily on gut bacteria (Zhang et al. 2015). The intestinal microbiota of humans is vast, varied, and ever-changing, containing around 100 trillion microorganisms, including at least 1000 distinct species (Li et al. 2022). The muscularis mucosae, lamia propria, and epithelial cells make up the human gut mucosa. It is colonized by 10^{14} microbes, which are ten times more numerous than human cells (Zhang et al. 2015; Tlaskalová-Hogenová et al. 2011; Singh et al. 2017). The large intestine contains by far the greatest number of microorganisms found in the human body (Pallister et al. 2016). Human health depends on the large and varied microbial community that lives in the intestines and has coevolved with our species (Kinross et al. 2011). *Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria* are the four bacterial phyla that dominate the gut microbiome of the majority of mammals (Ferreira et al. 2014; Cenit et al. 2014; Zhang et al. 2015; Kinross et al. 2011; Tlaskalová-Hogenová et al. 2011). An imbalance in the normal gut microbiota has been connected to larger systemic disease manifestations like obesity, type 2 diabetes, cardiovascular disease, allergic reactions, and neurogenerative diseases, as well as digestive issues such as inflammatory bowel illness and irritable bowel syndrome (IBS) (Aron-Wisnewsky et al. 2016; Sarkar et al. 2021; Laing et al. 2022; Jovel et al. 2018). The majority of the gut microbiota is found in the gastrointestinal tract, and it is essential to gastrointestinal disorders (Chen et al. 2023). There is mounting evidence that the microbiota determines the age

at which symptoms appear and that they are involved in chronic illnesses, such as IBD. Additionally, the microbiota may be a key target for treatment. While the selective use of probiotics, prebiotic foods, and/or supplements that less invasively change the gut flora may be more pleasant, fecal transplants have in fact been proposed for IBD and other disorders (Laing et al. 2022).

Early gut microbiome and chronic illness associations

There is evidence that the gut microbiome affects numerous physiological, metabolic, and immunological processes over the last ten years. The effect that the intestinal microbiome has in early life is therefore the subject of extensive research (Sarkar et al. 2021). Human gut microbiota has been linked to an apparently growing variety of illnesses, disorders, and functional abnormalities. Numerous factors, including age, nutrition, and medication, are known to contribute to enhanced discrete and long-lasting inflammation, in addition to genetic susceptibility, trauma, and numerous stress factors (both physical and emotional) (Bengmark 2013). The subsequent segment concentrates on providing an overview of the correlations that have attracted the most attention: the potential association between the intestinal microbiota and (1) chronic gastrointestinal disorders like IBS and IBD, and (2) systemic metabolic disorders like type 2 diabetes and obesity (Jovel et al. 2018).

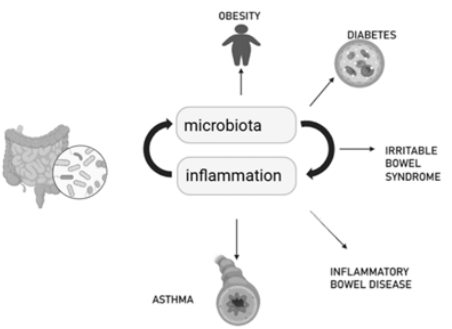

Fig.1: Role of microbiota in various chronic illness.

Inflammatory bowel disease (IBD)

The developed nations of Scandinavia, the United States, and Europe have the highest rates of inflammatory bowel disease (IBD) (Zhang et al. 2015). IBD disorders, which include Crohn's disease and ulcerative colitis, are hyperimmune, multifactorial conditions. With significant hereditary roots, these conditions are linked to inflammation and changes in the gut microbiota and metabolome (Ferreira et al. 2014; Sarkar et al. 2021; Durack et al. 2019). Anywhere along the GIT's length, CD is typified by an inflammatory pattern resembling cobblestones, with healthy tissue interspersed between the afflicted areas. Moreover, ulcerations that may cover the entire intestinal wall and cause fissures that could pierce the wall and affect other organs like the kidney or uterus are characteristic of it. Contiguous inflammation affecting mainly the intestinal wall's outer layers is the usual manifestation of UC. The rectum is where it usually starts and is mostly found in the colon (Jovel et al. 2018). Even in healthy individuals, certain microbiota composition is at risk due to genes known to be linked to IBD. Patients have been seen to have reduced microbial diversity and *Roseburia* abundance. Childhood and adolescence may be the initial stages of these illnesses, which can manifest as chronic, relapsing conditions that last a lifetime (Sarkar et al. 2021).

Irritable bowel syndrome (IBS)

The main symptoms of IBS include discomfort, pain, and changes in bowel movements. Despite having a complex etiology, new research on the pathophysiology of IBS has shown that changes in the normal gut microbiota may contribute to the syndrome's low-grade intestinal inflammation (Vijay et al. 2022; Jovel et al. 2018). It is estimated that 10% to 20% of adults and adolescents worldwide suffer from IBS. IBS is believed to have a complex etiology, while the precise cause is unknown. It is believed to develop due to a combination of genetic factors, GIT motor dysfunction, visceral hypersensitivity, infection, inflammation, immunity, and psychopathological causes (Jovel et al. 2018).

Table 1: Etiological classification of symptoms of IBS (Jovel et al. 2018).

IBS Subtype	Characteristics
IBS with constipation (IBS-C)	• More prevalent in women • Up to one-third of cases • Hard stools >25% of the time and loose stools <25% of the time
IBS with diarrhea (IBS-D)	• Up to one-third of cases • more common in men • loose stools >25% of the time and hard stools <25% of the time
Mixed IBS (IBS-M)	• Over 25% of the time, both hard and soft stools • Approximately one-third to half of cases
Unsubtyped IBS	• Stool consistency is not atypical enough to satisfy the requirements.

Obesity

The prevalence of obesity has skyrocketed, surpassing 20% in the majority of western nations. It is distinguished by an excessive or aberrant buildup of fat that has a detrimental impact on health. Lowered life expectancy and/or increased health issues, including heart disease, type 2 diabetes, obstructive sleep apnea, some cancers, and osteoarthritis, are caused by such disorders (Ferreira et al. 2014). The gut microbiota's composition is believed to play a role in the development of obesity, and the extensive use of antibiotics in the treatment of infectious disorders as well as industrial exposure to antibiotics can contribute to the development of obesity (Sarkar et al. 2021). The development of obesity is a complicated process that mostly combines genetic predisposition, inactivity, and excessive food and energy intake (Ferreira et al. 2014). Studies in humans and animals have linked obesity to various alterations in the composition of the gut microbiota as well as a decrease in bacterial diversity.

It has been noted that obese people have higher amounts of *Firmicutes* and lower levels of *Bacteroidetes* than lean people (Cenit et al. 2014; Sarkar et al. 2021; Zhang et al. 2015).

Diabetes

Obesity from a high-fat diet is linked to inflammation, which ultimately results in the development of insulin resistance and type-2 diabetes (T2D). The metabolic disorder known as diabetes has been a major global concern and has been found to be closely linked to gut flora. Diabetes can be divided into two types: type-1 diabetes (T1D) and type 2 diabetes. Diabetes is a result of alterations in gut flora (Murphy et al. 2015; Zhang et al. 2015). T-cells destroy the pancreatic beta cells that produce insulin, causing type 1 diabetes (T1D), an autoimmune condition (Cenit et al.2014; Sarkar et al. 2021). Type 2 diabetes is a complicated condition that is impacted by both environmental and genetic factors. It has grown to be a significant global public health issue (Jovel et al. 2018). Compared to healthy controls, those with type 2 diabetes have a different composition of gut bacteria. But it's unclear if these changes are a cause or a consequence, so more research is needed. Of the frequently reported results, T2D was negatively correlated with the genera *Bifidobacterium, Bacteroides, Faecalibacterium, Akkermansia, and Roseburia,* whereas T2D was positively correlated with the genera *Ruminococcus, Fusobacterium, and Blautia* (Vijay et al. 2022). The two helpful genera most commonly mentioned in T2D research are Bacteroides and *Bifidobacterium* (Gurung et al. 2020). The century's greatest epidemic is thought to be diabetes. Chronic hyperglycemia brought on by improper insulin secretion, function, or a combination of the two is a hallmark of the metabolic illness group known as diabetes. As an anabolic hormone, insulin plays a crucial role in the metabolism of proteins, fats, and carbohydrates (Sarkar et al. 2021).

Atopic asthma

Chronic asthma is characterized by increased mucus production, eosinophilia, and excessive contraction of the smooth muscle of the airways (known as airway hyper

responsiveness, or AHR). A Th-2-type inflammatory condition is asthma (Ferreira et al. 2014; Bora et al. 2023). There are other types of asthma, including nocturnal occupational asthma, non-allergic asthma, and pregnant asthma (Bora et al. 2023). In recent decades, the prevalence of asthma has rapidly increased in industrialized nations, especially among pediatric populations. This growth is believed to be attributable to changing environmental exposures associated with Western lifestyles rather than only genetic risk factors (Durack et al. 2019). The immunology of the airways is significantly impacted by the gut microbiome. Since genetic polymorphisms and environmental variables are important in the diagnosis and treatment of asthma, it is crucial to take the host microbiota's composition into account as well (Ferreira et al. 2014).

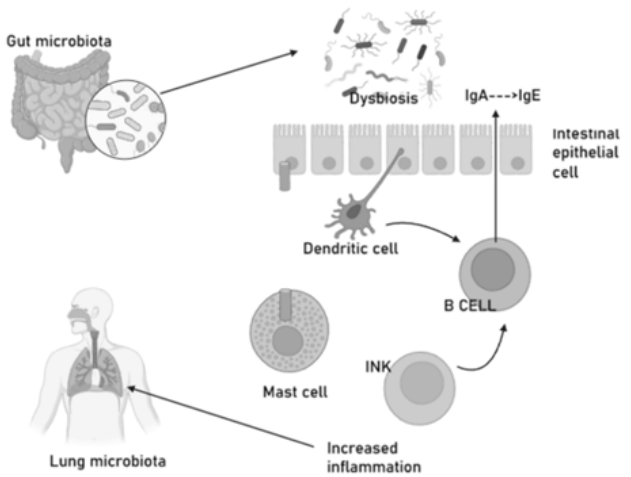

Fig.2: Role of gut microbiome in atopic asthma.

Implications of gut microbiota modification for therapy

The gut microbiota may have a therapeutic role in human health, which has given rise to therapeutic strategies like

bioecological management and bacteriotherapy. According to these beliefs, prebiotics, probiotics, and synbiotics, as well as the host's pre-morbid gut microbiota, may improve human health by modifying intestinal floral populations (Durack et al. 2019; Jovel et al. 2018). Protozoa, viruses, fungi, bacteria, and archaea make up the gut microbiota, which interacts with the host to affect its physiology and general health. When there are erratic changes in the gut ecology, GM modulation is detrimental. Probiotics led to a high-fat diet, which altered the GM structure. GM modification alleviates a number of health issues (Bora et al. 2023). A clear possible intervention that could affect the meta-organismal pathway is altering dietary nutrient intake (Tang et al. 2014).

CONCLUSION

Evolution of the human gut microbiota during the course of a person's life and seem to be crucial for both health and illness. The specific role of the gut microbiota in these diseases is unknown, but a dysbiotic state of the gut microbiota is increasingly understood as an environmental factor that interacts with a host's metabolism and plays a role in pathological conditions, including gut-related IBS and IBD as well as systemic conditions like obesity, diabetes, and atopy. Chronic inflammatory disease has been the subject of extensive investigation due of the disease's renewal and the uncertainty surrounding its beginning timing and mechanism. The results demonstrate that the development and resolution of persistent inflammation are inextricably linked to the equilibrium of intestinal microecology. Dietary and pharmaceutical intake, a sound genetic metabolism, and a healthy lifestyle are the main factors that guarantee this equilibrium.

FUTURE PROSPECTS

Over the past decade, there has been a notable surge in the scientific and clinical significance of the role of the complete microbiome in health and illness. The human immune system is now clearly impacted by alterations in gut microbiota, which can influence the onset of inflammatory and autoimmune disorders both inside and outside the gut. Despite the amazing

complexity of the gut's microbial communities, new options to define the human gut microbiota without performing cultures have been made possible by developments in next-generation sequencing and enhanced bioinformatic techniques. In order to improve cardiometabolic and inflammatory health outcomes, it is very likely that dietary therapies particularly aimed at raising specific bacterial metabolites will be developed within the next five years. The accumulation of slowly evolving, chronic pathophysiological alterations that have their roots in early life is the cause of many of the diseases currently thought to be connected to the early gut microbiota. Future tactics include converting this growing corpus of data into treatments that disrupt and modify the early gut microbiota.

Conflict of interest

There is no conflict of interest related to the study.

Author contributions

Acquisition and interpretation of data is done by Mushkan and Sounak Chakraborty. Conception, design and revising of the article are done by Rupesh Dutta Banik.

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Role of *Staphylococcus aureus* in skin and soft tissue infection

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ABSTRACT

Staphylococcus aureus is a Gram-positive, facultative anaerobic bacteria that penetrates human skin and soft tissues. Skin and soft tissue infections (SSTI) triggered by *Staphylococcus aureus* are growing increasingly prevalent globally, in hospitals moreover, the community. Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are widely reported in hospital and community contexts, and they might also consist of additional pathogenic bacteria and fungi. *S. aureus* generates almost one-third of severe bacterial skin and tissue infections, especially methicillin-resistant *Staphylococcus aureus* (MRSA). Its ability to remain in the skin, either commensally or during an infectious phase, is greatly affected by its cellular structural makeup and population diversity. It causes tissue infection when the skin or mucosal membranes are compromised, resulting in numerous illnesses such as boils, tumors, and cysts. SSTI can range from minor epidermal infections to life-threatening necrotizing fasciitis. *S. aureus* was sensitive to most of antibiotics, including Tetracycline, penicillin, ciprofloxacin, doxycycline, and azithromycin, Clindamycin, and chloramphenicol. This study focuses on the mechanism of this bacterium in the context of infection and available treatment options.

***Keywords**: Staphylococcus aureus, skin and soft tissue infections (SSTI), Methicillin-resistant Staphylococcus aureus (MRSA), antibiotics, mechanism, treatment options*

INTRODUCTION

Among the many dangerous enemies in the field of infectious diseases is *Staphylococcus aureus*, that frequently lives on healthy human skin, noses, and mucous membranes. This resilient organism has the alarming ability to develop antimicrobial resistance, either through spontaneous mutations or the acquisition of genes used by other bacteria to develop resistance, thereby becoming a leading source of human infections worldwide (Linz et al. 2023). Renowned for its adaptability, *S. aureus* invades human skin as part of the commensal flora, often resulting in various infections. *S. aureus* is The most prevalent bacterial infection linked to Skin and Soft Tissue Infections (Mariani et al. 2023). SSTIs are the most prevalent infectious condition requiring medical treatment. Folliculitis is a minor variety, but necrotizing fasciitis is life-threatening. *S. aureus* predominantly manifests in SSTIs such as impetigo, wounds, furuncles, burns, and abscesses, as well as respiratory and urinary tract infections (Mahakalkar et al. 2020). Healthy skin is dry, has a low pH, constantly sheds its upper layer, and produces antimicrobial peptides, creating a hostile environment for bacteria. However, *S. aureus* can overcome these defences due to its cellular structure and population heterogeneity, allowing it to colonize the skin. It adapts to stressors present in conditions like atopic dermatitis and chronic wounds in diabetes patients (Gehrke et al. 2023). *S. aureus* causes skin infections through the deployment of various virulence factors. These include cytolytic proteins, super-antigenic factors, cell wall-anchored proteins, and molecules designed for immune evasion. The immune response to SSTIs begins with the action of keratinocytes and neutrophils as initial responders, which are later supported by dendritic cells and T-lymphocytes throughout the entire infection (Linz et al. 2023). *S. aureus* can cause infections anywhere on the body with pilosebaceous units. However, it is most commonly isolated in cases of hair loss, which typically

affects the face. Folliculitis, the inflammation of a hair follicle, usually presents as clusters of red papules less than 5 mm in diameter. The risk factors for SSTIs, particularly those strains sensitive to methicillin, are well-documented. These include various co-morbidities such as diabetes mellitus, Peripheral vascular disease, renal disease, cardiovascular disease, chronic wounds, immunosuppression, intravenous drug use, and the existence of abscesses. Children are at a higher danger of creating skin infections if they have eczema, chronic health conditions, nail-biting habits, or should they be Black. Other danger factors include possessing a household member with a skin infection within the past year or being colonized with methicillin-resistant *Staphylococcus aureus* (MRSA) (Linz et al. 2023).

MATERIALS AND METHODS

Searching PubMed, PubMed Central, Google, and published research and review articles worldwide on the environmental pollutants generated by the leather industries and the microbial bioremediation technique to eliminate the pollutants for a cleaner world is how the relevant material for this review paper was discovered. Only published data was taken into account; ambiguous exposure statements were not. A component of these inclusion criteria is information gathered from reliable publications on the topic. The only language included in the study was English.

Virulence factors

Many virulence factors, such as cytolytic proteins, super antigenic factors, molecules useful for immune evasion, and cell wall-anchored proteins, are produced by the bacterium *S. aureus* and are believed to be significant for skin infections. Protein A and clumping factor are examples of adhesins that help cells and tissues adhere to one another. Alpha-toxin and Panton-Valentine Leukocidin (PVL)are two examples of toxins that can harm tissue and induce necrosis. Enzymes capable of breaking down host tissues and aid invasion include lipases and proteases (Kobayashi et al. 2015).

Pathogenesis

Colonization

The skin and mucous membranes are the first places where this opportunistic infection settles. In places like the skin or nose, where the bacteria can find the perfect conditions to flourish, this colonization frequently takes place. Surface proteins that aid in adhesion to host tissues, such as clumping factors and fibronectin-binding proteins, are produced as the main mechanism of colonization. The bacteria are able to take root because these proteins bind them to the skin and mucosal surfaces. Colonization typically has no symptoms, but if the skin barrier is weakened, it can lead to infection. A number of variables, including skin conditions, immune system function, and personal hygiene, might affect how much colonization occurs. One of the main risk factors for future infections is nasal carriage of *S. aureus*.

Invasion

The invasion phase begins when *S. aureus* breaches the skin barrier, typically through minor trauma, such as cuts, abrasions, or insect bites. The bacteria exploit these breaches to penetrate deeper into the skin and underlying tissues. Several virulence factors facilitate this invasion process:

Enzymes: *S. aureus* secretes a variety of enzymes, including proteases, lipases, and hyaluronidases, that degrade the structural components of the skin. This enzymatic activity breaks down proteins, lipids, and hyaluronic acid, creating pathways for the bacteria to invade.

Toxins: The production of cytolytic toxins, such as alpha-toxin and Panton-Valentine Leukocidin (PVL), helps in lysing host cells, leading to harm to tissue, and facilitating bacterial spread. Alpha-toxin forms pores in the membranes of host cells, leading to cell lysis and tissue necrosis.

Adhesins: The existence of adhesins, which are proteins found on the surface and bind to host extracellular matrix components, allows the bacteria to firmly attach to and invade host tissues.

During this phase, the host's innate immune response is activated. Neutrophils, the first responders, attempt to contain the infection through phagocytosis and the release of antimicrobial substances. However, *S. aureus* has evolved mechanisms to evade these defenses, including the production of protein A, which binds to the Fc region of antibodies, preventing opsonization and phagocytosis.

Proliferation

Once *S. aureus* has successfully invaded the tissues, it begins to proliferate and spread, causing significant tissue damage and inflammation. The proliferation phase is characterized by the rapid multiplication of bacteria and the dissemination of the infection to surrounding tissues. Several factors contribute to this process:

Iron Acquisition: *S. aureus* requires iron for growth and survival. It produces siderophores, which are small, high-affinity iron-chelating compounds that scavenge iron from host proteins. This ensures a steady supply of this essential nutrient.

Biofilm Formation: Biofilms can arise from *S. aureus*, which are structured groups of microorganisms contained within a self-produced extracellular matrix. Biofilms provide a protective environment that enhances bacterial survival and resistance to host immune responses and antibiotics (Gehrke et al. 2023).

Immune Evasion: *S. aureus* employs several strategies to evade the host's immune system. It produces surface polysaccharides that inhibit phagocytosis and secretes factors that inhibit complement activation. Additionally, the bacterium may alter the immunological responses of the host by releasing proteins that obstruct neutrophil recruitment and function.

The proliferation of *S. aureus* brings about the release of additional virulence factors, further exacerbating tissue damage and inflammation. The host's immune system continues to respond, with additional immune cells, such as macrophages, dendritic cells, and T cells, being recruited

toward the infection location. Despite these efforts, *S. aureus* can persist and establish chronic infections, leading to abscess formation and other complications.

Treatment options

Skin and soft tissue infections (SSTIs) cover a variety of illnesses, from simple superficial infections to potentially fatal disorders like necrotizing fasciitis. SSTI treatment is determined by the kind of infection, causative organisms, disease severity, and patient-specific factors such as comorbidities and allergies.

Oral therapy: It is typically used for *S. aureus* SSTIs, with parenteral therapy reserved for severe cases. Treatment options include cephalosporins and penicillin agents like oxacillin for MSSA and vancomycin for MRSA (Linz et al. 2023).

Empirical therapy: It begins based on the most likely infections and local antimicrobial susceptibility patterns. Non-purulent cellulitis (lack of purulent material) is typically caused by beta-hemolytic *Streptococcus sp*. Mild instances is possible to cure using oral antibiotics such cephalexin (500 mg every 6 hours) and dicloxacillin (500 mg every 6 hours). Clindamycin (300 mg every 8 hours) is advised in situations of severe penicillin allergy. Vancomycin (10-15 mg/kg every 12 hours) may be used for severe infections or those that do not respond to early treatment.

Purulent Infections: *Staphylococcus aureus*, including methicillin-resistant *Staphylococcus aureus* (MRSA), is frequently involved in purulent SSTIs, such as abscess and furuncles. The foundation of abscess treatment is drainage and incision (I&D)2. Antibiotics that target MRSA, such as clindamycin, doxycycline, or trimethoprim-sulfamethoxazole, are used empirically to treat cellulitis with purulence.

Treatment options for impetigo, which is caused by *Staphylococcus aureus* and *Streptococcus pyogenes*, include topical mupirocin ointment for mild cases and oral antibiotics like cephalexin for more severe ones. *Staphylococcus aureus* is usually the cause of folliculitis, which is treatable with topical

antibiotics and warm cloths. Necrotizing fasciitis: This serious infection necessitates beta-lactams and vancomycin, among other broad-spectrum antibiotics, as well as rigorous surgical debridement (Stevens et al. 2015).

Adjunctive therapies: In the treatment of SSTIs, adjunctive therapies—like wound care and supportive measures—are essential. Proper wound care, which includes washing and changing dressings, encourages healing and helps avoid subsequent infections (Marcelin et al. 2018).

CONCLUSION

S. aureus is the predominant pathogen causing skin and soft tissue infections (SSTIs). While hospitalization rates for *S. aureus* SSTIs are declining in the U.S., these infections remain a significant healthcare challenge, leading to considerable morbidity and mortality. In 2019, *S. aureus* was the leading bacterial cause of death in 135 countries, contributing to over a million deaths. Most *S. aureus* SSTIs are manageable with oral antibiotics, but they can escalate into severe infections requiring intravenous therapy. With the rise of antimicrobial-resistant strains, experimental treatments like bacteriophage therapy are being explored. MRSA alone was in charge of over 100,000 deaths globally in 2019.

Additional investigation is required to understand host factors and pathways that influence responses to *S. aureus* infections, genetic susceptibility, and virulence factors. New therapeutics targeting bacterial processes like biofilm formation are promising. Challenges in vaccine development, such as the immune-modulating properties of protein A, also need to be addressed. In summary, *S. aureus* is highlighting the need for ongoing research and innovative treatment strategies.

Conflict of Interest

There is no conflict of interest related to the study.

Author contributions

Acquisition and interpretation of data is done by Prakriti Patra. Conception, design and revising of the article are done by Rupesh Dutta Banik.

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Nano-enabled theranostics for brain infections: Exploring the potential of nanoparticles and graphene

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ABSTRACT

Study aiming at formulating viable treatments for neurological disorders is intensifying in the context of their increasing prevalence. Common neurological illnesses that remain difficult to treat include ischemic stroke, glioma, neurodegenerative diseases, and traumatic brain injury. So, the blood–brain barrier (BBB), which is a very selective semipermeable membrane, keeps small molecules, proteins, and oligonucleotides from working as medicine. The blood–brain barrier (BBB), which encases the brain, protects its intricate microenvironments. Due to their ability to selectively transport pharmaceuticals across the blood-brain barrier, multifunctional nanomaterials have emerged as appealing carriers for targeted drug delivery to the brain. Graphene-based nanomaterials exhibit exceptional optical, mechanical, and biological properties. Due to their conductivity, they can serve as a substrate in tissue engineering, facilitate neural connections, and guide the growth and differentiation of neurons. Consequently, graphene-based nanomaterials represent an emerging domain in regenerative medicine. Active targeting utilizes nanostructures with exceptional receptor selectivity and targeting precision, making them ideal payloads for traversing the blood-brain barrier. Microorganisms can efficiently traverse the blood-brain

barrier and assimilate nanoparticles exhibiting biomimetic characteristics. This study focuses on a wide range of traditional diagnostic methods as well as important nanoparticles, including polymers, lipids, gold, and carbon nanotubes, that can cross the blood-brain barrier and help find meningitis and brain infections early. Furthermore, it highlights medication delivery methods utilizing nanotechnology for the study of cerebral infections, including meningitis. The construction and function of the BBB are highlighted in this study, along with the potential of nanotechnology to transcytoses into the brain, which may aid in the treatment of neurological disorders.

Keywords: *Nanoparticles, blood-Brain Barrier (BBB), graphene-based nanomaterials, neurodegenerative diseases, drug delivery*

INTRODUCTION

Brain infections can be caused by bacteria, fungus, protozoa, parasites, and prostate viruses. Acute meningitis is frequently caused by bacteria (*Streprococcus pneumoniae*, *Neisseira meningitidis, Streprococcus agalactiae, Haemophilus influenzae,* and *Listeria monocytogenes*) and viruses (mostly Enteroviruses HIV, herpes simplex viruses (HSV), and mumps virus) (Tunkel et al., 1993). Brain infections frequently affect other areas of the central nervous system, such as the spinal cord. Infection can also result from inflammation of the brain and spinal cord's tissue layers (Woehrl et al. 2011). Necrosis, reduced blood and cerebrospinal fluid (CSF) flow, and compromised central nervous system (CNS) functions are all possible outcomes of meningitis. Every age group is susceptible to infectious meningitis, a dangerous central nervous system illness that involves microbial inflammation of the meninges. Bacterial meningitis has an extremely high death and morbidity rate, even following antibiotic and anti-inflammatory treatment. Therefore, bacterial meningitis treatment has been developed in recent decades to both eliminate the invasive germs and break down the resulting inflammatory reaction. The European Federation of Neurological Societies (EFNS) guidelines state that the current therapy of choice

for non-infected adult patients with bacterial meningitis is a combination of corticosteroids and bacteriolytic E-lactam antibiotics (Chaudhuri et al. 2008). Lumbar puncture (LP), also known as spinal tap, is the current diagnostic method for meningitis. During this technique, a tiny volume of CSF is obtained for testing for bacterial and viral infections. Post-LP headache, infection, hemorrhage, cerebral herniation, back discomfort, late-onset epidermoid tumors of the thecal sac, and mild neurologic symptoms including radicular pain are among the possible consequences (Engelborghs et al. 2017). Future individuals with bacterial meningitis contracted in the community may find antibody adjuvant therapy to be a good choice. The development of nanotechnology has demonstrated that the diagnosis of brain infections and meningitis can be effectively treated with nanosensors and nanomaterials (Kischkel et al. 2020). Some characteristics of the nanoparticles include their easy functionalization, simple synthetic method, ability to cross cell membranes, increased drug release at the site of infection due to a higher surface-to-volume ratio, ability to kill the pathogens by inhibiting multiple bacteriological targets, and, most importantly, biological safety (Barani et al. 2021).

It is important to pay particular attention to the blood-brain barrier (BBB). Many therapeutic substances cannot enter the brain from the circulatory system because of the BBB. This is mostly because of ATP-binding cassette proteins called ABC transporters, which make treating brain illnesses difficult (Pereira et al. 2017). There are a number of ways to transport drugs to the brain, but the majority have drawbacks such high risk, high expense, and incompatibility (Ahlawat et al. 2020). In numerous brain-specific treatments, carbon nanotubes (CNTs) have shown great promise as therapeutic agents and nanocarrier systems (Wong et al. 2013; Li et al. 2011). More specifically, CNTs were able to create interfaces with neurons and readily traverse the blood-brain barrier (Ma et al. 2018; Kafa et al. 2015). CNTs do have certain drawbacks, though, such as their toxicity, the inability to manage their length, and their high production costs (Liu et al., 2013; Francis et al. 2018). However, graphene-based materials and carbonaceous zero-dimensional carbon nano-onions have already been

shown to have anti-neurodegenerative properties (John et al. 2015; Mendonca et al. 2015; Pakhira et al. 2016; Plonska-Brzezinska 2019). However, there are several drawbacks to employing those minerals in their pure forms, such as the inability to create well-dispersed aqueous solutions because of their hydrophobic character. For these materials to be used in biomedical applications, additional surface modification is also required (Wu et al. 2019). Here, we describe the application of graphene quantum dots (GQDs) as brain drug nanocarriers that are appropriate for bioimaging because of their capacity to dissolve protein clumps that lead to neurodegenerative disorders and traverse the blood-brain barrier (Gómez et al. 2024).

Nanomaterials and nanoparticles for the treatment of neurological disorders:

Polymeric nanoparticles:

In order to bind, adsorb, dissolve, and encapsulate medications or therapeutic chemicals, polymeric nanoparticles (NPs) are solid colloidal particles that incorporate macromolecular components. Drug delivery methods for treating neurological diseases frequently use degradable polymeric nanoparticles (NPs) with a diameter of 10–100 nm. The two variable units of these particles are nanospheres and nanocapsules (Fornaguera et al. 2016; Das et al. 2016; Soppimath et al. 2001; Pisani et al. 2009; Kreuter 2014). Coreshell NPs make up nanocapsules, while homogeneous matrices are seen in nanospheres. These particle sizes allow for fine-tuning to achieve desired characteristics, such as the preservation of active compounds with simple administration and the penetration of pharmaceuticals into the target cells with increased efficacy and efficiency at cheap production costs (Jain 2012; Neha et al. 2013; Lee et al. 2009).Furthermore, the appropriate rate of disintegration and the particles' ability to penetrate the blood-brain barrier and enter the central nervous system make them effective (Wilson 2009).

Solid Lipid Nanoparticles

For brain targeting, SLNs are seen as an appealing colloidal drug delivery technology. The use of SLNs for targeted

medication delivery in the brain is restricted by their accumulation in the reticuloendothelial system. The lipid matrix has a distinct size, is solid at room temperature, and has the advantage of being a nanocarrier, which improves drug release and stability without having harmful effects on the tissue (Roney et al. 2005). The SLNs have greater advantages in terms of scale-up feasibility and reproducibility through the use of numerous techniques. For other formulations without organic solvents, it is likewise a good choice. Additionally, this lessens the possibility of lingering contamination. These characteristics make SLN one of the most promising drug delivery methods for the treatment of cancer and a variety of neurological diseases (Cacciatore et al. 2016; Sozio et al. 2015; Gastaldi et al. 2014).

Gold Nanoparticles

Better biocompatibility, simple surface functionalization, and facile and efficient transport to target cells and tissues are only a few of their many noteworthy qualities. According to some research, modest microwave field exposure in brain tissue can assist gold nanoparticles (NPs) break down and dissolve Aβ fibrils and plaques, which can be used to treat AD illness. Aβ fibrils and plaque buildup in the brain are major symptoms of AD that can be avoided or eliminated (Siddiqi et al. 2017).

Carbon Nanotubes

Iijima made the discovery of the carbon nanotube (CNT) in 1991 (Iijima 1991). Ultra-light weight, high flexibility, low deposition, low cost, high capability, ultra-strong, and inert with electrical and thermal conductivity are only a few of their numerous advantageous qualities. Due to its unique and beneficial qualities for treating neurological conditions, such as AD, PD, and ischemic stroke, it has currently become one of the most promising novels NMs (Komane et al. 2016; Kakkar et al. 2015; Folch et al. 2016). Numerous conditions, including cancer, rheumatoid arthritis, osteoporosis, and bone implants, have been successfully treated with CNTs as drug delivery vehicles in vivo (Kakkar et al. 2015; Malarkey et al. 2007; Siddiqi et al. 2018).

Advantages of metal oxide-based nanoparticles

These NPs, or nanomedicine, can be utilized to diagnose life-threatening illnesses because of their amazing qualities, which include antibacterial action, anti-insecticidal activity, and nontoxicity. Because cerium nanoparticles are unstable in living things, their application is restricted, despite the fact that they can aid in the fight against cancer and AD (Singh et al. 2021). According to Kievit and Zhang (2011) and Tomitaka et al. (2015), iron oxide nanoparticles also demonstrated excellent qualities in the therapy of GBM. However, further research is required to develop metal and metal oxide nanoparticles for effective treatment of central nervous system illnesses (Singh et al. 2021; Annu et al. 2022).

Structure and function of the BBB in healthy and diseased brains

For functional synaptic signaling pathways that govern motor skills, memory, and several other activities, the BBB shields the brain's microenvironment from external effects (Dias et al. 2022). BBB penetration while minimizing off-target effects is necessary for designing the perfect material for brain therapies. Target selection, cell entrance, even distribution within the brain, and exocytosis from cells and the brain following therapeutic activity are other crucial factors. The brain's normal equilibrium depends on the blood-brain barrier. Encircling cerebral capillaries and microvessels, it is made up of endothelial cells that interact closely with astrocytes and pericytes. Tight junctions (TJs) and adherens junctions (AJs), which form the junction of endothelial cells, form a barrier that envelops the brain (Wilhelm et al. 2014). 99 percent of the brain's capillary and neuronal terminals' abluminal surface is encircled by astrocytes (**Fig. 1**) (Pardridge 1999). The BBB is frequently compromised in neurodegenerative illness, stroke, and traumatic brain injury (TBI). This allows harmful substances to enter the body and alters regular metabolic processes, which results in several defects in the function of astrocytes, neuron cells, and microglia cells (DiPardo et al. 2017). Designing NP-based disease treatment systems requires an understanding of the structure of the blood-brain barrier (McLoughlin et al. 2024).

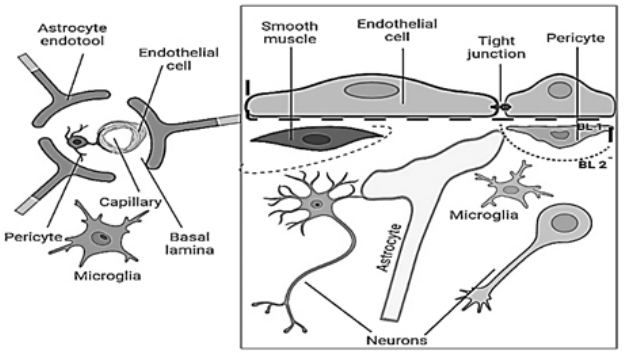

Fig 1. Blood–brain barrier's structure and composition.

Neurodegenerative diseases and blood–brain barrier

Transcellular-lipophilic diffusive routes, paracellular-tight junctions, transport proteins, receptor-mediated endocytosis, and adsorptive transcytosis are some of the physiological pathways by which substances can pass through the blood-brain barrier (Gonzalez-Carter et al. 2020).

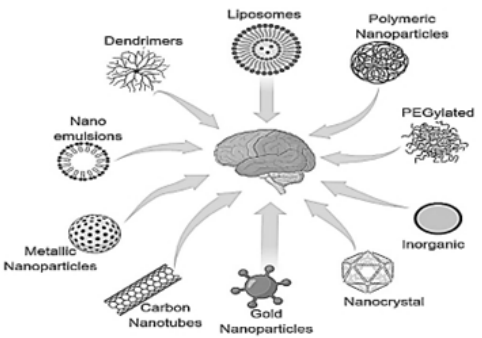

Fig 2. Nanotechnology-based drug delivery systems approach on treatment of CNS disorders.

To improve CNS medication delivery, several transport pathways have been investigated. Transient BBB disruption is one of the more specialized neurosurgical drug delivery techniques that may be useful in treating CNS disorders. Among the different therapeutic approaches, therapeutic systems based on nanomedicine offer a fresh approach to delivering beneficial substances with encouraging pharmacokinetics to boost the effectiveness of the medications now on the market to treat CNS diseases (Pardridge 1998; Nguyen et al. 2021).

Antimicrobial activity of graphene materials

Role of material physicochemistry-

Nanotoxicological effects on microbial cells are referred to as the antibacterial activity of nanomaterial systems. The antimicrobial activity of GMs is therefore dependent on their nanoscale structures and physicochemical properties, including dimensions, solubility, dispersability in solutions (in relation to surface charges and aggregation behaviors), capability in producing oxidative species, and existence of capping agents in solutions, as has been noted for the nanotoxicity of almost all nanoparticles (Buzea et al. 2007; Ripp et al. 2011). A wide range of structurally and physicochemically diverse materials are referred to be GMs. These materials include pristine graphene (PG), few-layer graphite platelets (GPs), oxidized and reduced variants of PGs and GPs (e.g., GO and rGO), and other compounds linked to graphene, such as GQDs. (Bianco et al. 2013; Wick et al. 2014; Zhen et al. 2018).

Utilization modes of graphene materials

Since antimicrobial GMs are a relatively new field, most of the research that has been published so far is proof-of-concept research categorized into four main themes based on the intended use areas: (i) antimicrobial colloids; (ii) contact-killing surfaces; (iii) release-killing surfaces; and (iv) light-activated disinfectants (also known as photothermal/ photodynamic inactivation) (Karahan et al. 2018).

Pathophysiology of meningitis:-

The CNS is protected from blood-borne pathogen invasion by the effective BBB and CSF barrier, as well as an outer layer of leptomeninges and the skull. Therefore, for the CNS infections to enter the brain, they need either a biological host defense gauntlet or an outer membrane defect (such as purulent mastoiditis, post-traumatic or post-neurosurgical dura leak). Multiple pathogen-host interactions that concurrently result in mucosal colonization, invasion, and survival within the intravascular space, as well as blood-brain/CSF barrier crossing, are necessary for effective CNS invasion (Herold et al. 2019).

Application of nanoparticles for improved therapeutics of meningitis

The best chance of obtaining superior meningitis therapy is through distribution approaches based on nanotechnology (Barani et al. 2021). How easily nanoparticles penetrate the skin or barrier depends on their size, charge, and general structure (Sun et al. 2020). The BBB's tightness is mostly caused by the vascular layers of brain capillary endothelial cells, which are connected side by side by adherens and tight junctions. Transcytosis via receptors is the most common way that NPs enter the brain. Using ligands such as transferrin, lactoferrin, antibodies, or surfactants like tween 80, the functionalized NPs can be created. NPs can be released at the opposite region of the membrane when ligands for NPs connect with their receptors on the luminal side of endothelial cells, causing plasma membrane invasions and pinch-free vesicle formation. Efflux pumps may decrease the quantity of NPs that are maintained in the brain parenchyma (Sharma et al. 2021).

Drug delivery methods for the brain

Invasive approach

By breaching the blood-brain barrier, this mechanical method delivers the medication directly into the brains. For the administration of intracranial hemorrhage medicines and intracerebroventricular (ICV) injection, brain surgery is

required (Stenehjem et al. 2009; Gabathuler 2010). The BBB is disrupted for drug diffusion when the endothelial tight junction collapses (Inamura and Black 1994; Stenehjem et al. 2009).

Pharmacological approach

This empirical methodology is based on the spontaneous inactive passage of medications over the blood–brain barrier (Stenehjem et al. 2009; Gabathuler 2010). These substances can pass through the blood–brain barrier on their own because of their small molecular size, low hydrophobic interaction ability, and water solubility (Hsiung et al. 2003). This approach, which enhances drug transport across the blood-brain barrier, also includes physiological modifications, such as a reduction in the quantity of polar functional groups (Pardridge 1998; Rhaman et al. 2022).

Advantages of NP's for brain therapy:-

NPs Have Small Particle Size That Facilitates Their Penetration Across the BBB:

The primary obstacles to treating brain diseases and disorders have been overcoming the blood–cerebrospinal fluid (CSF) and blood–brain barrier (BBB). P-gp carefully mediates the efflux of materials across the blood-brain barrier; therefore, its downregulation is linked to the development of tumors and neurological diseases (Henson et al. 1992; van Assema et al. 2012; Jeynes and Provias 2013). P-gp inhibition enhances a drug's ability to pass the blood-brain barrier and produce its intended effect (Jablonski et al. 2014).

NPs Have Low Toxicity and Can Be Used To Improve the Toxicity of Conventional Drugs in Their Targeted Cells:

The cytotoxicity of the majority of medications affects their therapeutic efficacy. When compared to traditional therapy, NPs cause significantly less brain toxicity. For instance, intranasal delivery of PLGA NPs has been shown to be extremely successful in transporting the mitoNEET ligand inhibitor NL-1 in a brain ischemia/reperfusion paradigm without causing any harm (Saralkar et al. 2020). Additional research has also demonstrated that encapsulating cytotoxic

drugs like sorafenib (an anticancer medication), amphotericin B (an antifungal medication), and Thioridazine (an antipsychotic medication) into NPs significantly raises their toxicity index by improving drug solubility, bioavailability, and sustained release (Tang et al. 2015; Vibe et al. 2016; Li et al. 2020).

Application of NPs in the Treatment Of Brain Diseases and Disorders:-

Huntington's disease (HD), epilepsy, amnesia, ischemic stroke (IS), brain tumor, and amyotrophic lateral sclerosis (ALS) (Ngowi et al. 2021).

Challenges in Brain Immunotherapy Based on Nanomedicine:-

Only a small number of medications, including rivastigmine, memantine, galantamine, tacrine, and donepezil (Birks et al. 2015), are currently used to support the clinical treatment of neurodegenerative disorders (Lammers et al. 2011). The central nervous system (CNS) is the most complex and sensitive structure in the human body, and it is securely packed and sealed by the blood-brain barrier (BBB) and brain cell membrane (BCFB) (Furtado et al. 2018) (Erickson et al. 2013). Because of its complexity, there are many limitations in delivering targeted medications from the blood to the central nervous system (CNS). This includes a lack of information regarding the role of medications, their half-lives, or bio-presences for brain cells, their effects on the CNS, or the irregular and uncertain relationships between off-target medications and receptors and proteins. The unstable index of evaluated medications is made up of the patient's genotype, the complex pharmacology of certain medications, neurodegenerative symptoms, the long latency period and incompetence of medications, the subsequent development of infections, an incorrect number of medications, and a variety of medication reactions. Furthermore, the challenges are made considerably more challenging by issues pertaining to the brain (Fernández et al. 2018; Sharma et al. 2015; Guo et al. 2023).

Graphene-based Nanomaterials (GBNS) as multifunctional drug-delivery system for the nervous system

Chemical modification through the addition of hydroxyl, carboxyl, amino, or other functional groups is made possible by the vast surface area of GBNs (John et al. 2015). As an alternative, noncovalent functionalization (hydrophobic or π–π stacking interactions and hydrogen bonding) can be used to load medicinal compounds into GBNs (Georgakilas et al. 2016). Due to shortcomings in existing therapeutic techniques, such as the poor hydrophilicity of many anticancer drugs, the decreased accessibility of the central nervous system, and the unspecific toxicity of chemotherapy on healthy tissue, research efforts were mostly focused on developing new therapeutics for brain tumors (Arvanitis et al. 2020). In order to treat CNS malignancies with a high mortality rate in children, GBNs were used as nanocarriers (Kaatsch 2010). While the use of harsh treatments already in use can boost survival rates in certain situations (Weil et al. 2017), the long-term and late effects of treating children cancer can be severe and irreversible, resulting in cognitive dysfunctions, emotional disorders, and subsequent neoplasms (Castellino et al. 2014; Marusak et al. 2018; Izycka-Swieszewska et al. 2018). Neuro-oncology in children and adults may benefit from the use of graphene as a drug-delivery nanocarrier (Cellot et al. 2022).

Table 1. Most promising graphene-based nanomaterial applications for neural growth and central nervous system regeneration (Wang et al. 2020)

Graphene-Based Scaffold	Biomedical Applications	Main Results	References
rGO encapsulated on poly (l-lactic acid-co-caprolactone) microfibers (PLCL)	Development of 3D neural networks	Outgrowth of neurite and formation of oriented neuronal-like networks	(Wang et al. 2020)

Laser-Scribed rGO	Generation of micropatterned in vitro neuronal networks	Adhesion and survival of rat primary neurons and guide the subsequent elongation of neurites	(Lee et al. 2018)
Ginseng-rGO sheets	Neural stem cell (NSC) differentiation	Accelerated differentiation of neural stem cells into neurons	(Akhavan et al. 2014)
3D-graphene foam	NSC proliferation and cell fate decision	Enhancement of NSC proliferation through metabolic regulation	(Fang et al. 2020)
Silk/ GO micro/ nano-fibrous scaffold	Nerve regeneration	Enhancement of metabolic activity, Neuronoma NG108-15 cells proliferation and neurite outgrowth	(Magaz et al. 2021)

Clinical advancements in nanomaterials for neurological diseases

APOLLO: Transthyretin-mediated amyloidosis, a rare neurological illness caused by transthyretin misfolding, is presently being treated in phase three clinical trials using Patisiran ALN-TR02, an NP assembly made up of siRNA-loaded lipid NPs (Zhang et al. 2020). The results of the phase three trial were recently published (Quan et al. 2023). Another IONP, the Pulse NanoMed System, is scheduled to undergo a clinical trial (Chung 2023). A Phase 2 clinical trial for guided brain-directed stereotactic radiation therapy is presently underway using gadolinium-based NPs, with completion anticipated in 2026 (MD 2022). They can ascertain whether the NP improves the precision and efficacy of radiation therapy by contrasting stereotactic radiation with regular stereotactic radiation for brain cancers and brain metastases. In 2021, one study investigated enhancing the BBB transport of siRNA-conjugated gold nanoparticles for Bcl2L12 downregulation. Brain metastases from breast cancer and other cancer types

were treated with this medication in a phase 1/2a clinical trial (2B3-101) (Kumthekar et al. 2021; Aftimos et al. 2014; Brandsma et al. 2014; McLoughin et al. 2024).

Table 2. Treatment trials using Nanoparticles for brain diseases and disorders (Lackner et al. 2008)

Nanoparticles	Disease	Mechanisms	Effects	References
Silver Nanoparticles	Acute occlusive hydrocephalus	Ventricullitis caused by catheters is prevented	Enhanced the health of patients	(Lackner et al. 2008)
Magnetic iron oxide nanoparticles + reduced radiotherapy	Glioblastomia multiformie	Increased Caspase-3, heat shock protein, and programmed death ligand 1 levels suppress tumor growth	Enhanced the overall survival rate of patients	(Maier-Hauff et al. 2011; Grauer et al. 2019)
Ultrasmall magnetic iron oxide	Ischemic stroke	Activated macrophages	Targeted inflammatory cytokines more effectively	(Saleh et al. 2007)
Nano-curcumin + omega-3 fatty acid	Migraine	Intercellular adhesion molecule 1, TNF-a, and cyclooxygenase-2/ inducible nitric acid gene expression is suppressed	Relieved headaches	(Abdolahi et al. 2019; Abdolahi et al. 2017; Soveyd et al. 2017)

CONCLUSION AND FUTURE PERSPECTIVES

Conventional medication delivery techniques are unable to penetrate the brain's complex physiology. Thanks to the advancement of nanotechnology, medications for infections can now be administered by piercing the blood-brain barrier. Nanomaterials have showed a lot of promise due to their flexibility, safety, biodegradation, and biocompatibility. In the modern day, the use of nanoparticles to treat infectious brain diseases is a potential prospect due to recent advancements. By incorporating them into suitable nanostructures,

the molecules with decreased hydrophobicity can also be dispersed. Additionally, the anti-infectious substance can be directly administered through the bacterial surface receptors without posing a threat to the surrounding tissues. But before thinking about therapeutic applications, safety concerns must be addressed in light of the latest developments. In order to treat brain infections and nervous system failure, nanotechnology presents exciting answers to the primary diagnostic problems. The safe, efficient, and targeted delivery of biological substances, such as anti-inflammatory medications, for the diagnosis of infectious diseases, such as meningitis, is a promising use for nanoscale devices. Naturally, diagnostic tools are required to address certain issues, such as the ability to cross the blood-brain barrier, and developments in nanotechnology will improve the capacity to identify brain infections. Working successfully in clinical trials, which will address any potential nanotoxicological difficulties, is the next essential step in developing this subject. Until recently, a significant amount of research in this field has been carried out using different animal models.

Conflict of Interest

There is no conflict of interest related to the study.

Author contributions

Acquisition and interpretation of data is done by Tanisha Bhowmick, Dipti Das, Tiyasha Saha. Conception, design and revising of the article are done by Rupesh Dutta Banik.

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Advancements in modern biotechnological approaches for combating HIV infection

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ABSTRACT

Human immunodeficiency virus (HIV) continues to pose a major global health threat, affecting millions of individuals. While antiretroviral therapy (ART) has made substantial progress in managing the infection, a complete cure remains elusive. This review focuses on the latest advancements in biotechnological strategies aimed at combating HIV. Promising approaches include the development of therapeutic vaccines, monoclonal antibodies, and RNA interference. Therapeutic vaccines are being designed to enhance immune responses, while monoclonal antibodies, particularly broadly neutralizing ones, target viral replication. RNA interference, utilizing small interfering RNA (siRNA), is being explored to silence essential viral genes and reduce viral burden. Furthermore, gene-editing tools such as CRISPR-Cas9 offer potential for the targeted removal of HIV from infected cells. This review highlights the mechanisms and ongoing research surrounding these innovative technologies, evaluating their potential to overcome the challenges of current HIV treatments and bringing us closer to a cure.

Keywords*: Human immunodeficiency virus (HIV), antiretroviral therapy (ART), therapeutic vaccines, monoclonal antibodies, RNA interference, CRISPR-Cas9*

Introduction

HIV/AIDS is a major public health concern worldwide more than 30 years after HIV was identified as the cause of acquired immunodeficiency syndrome (AIDS). With over 2 million new infections and 1 million AIDS-related deaths annually, there are currently over 36 million individuals living with HIV (Auclair et al. 2018). HIV infection causes a significant reduction in host immunity through the death of $CD4^+$ T-cells, which throws off the balance between the host immune response and the human microbiome (Li et al. 2023). Although the primary method of transmission in adults is heterosexual, mother-to-child transmission (MTCT) during pregnancy, labor, and delivery, as well as postpartum breastfeeding, accounts for the majority of pediatric infections (Amin et al. 2021). HIV is a retrovirus with a lipid-based envelope that is around 100 nm in diameter. The envelope contains 72 trimers of the Env proteins, gp120 and gp41. Within the envelope is a conical capsid that contains two copies of positive-sense single-stranded RNA that code for structural and regulatory proteins. The HIV genome encodes several regulatory proteins, including the transactivator protein (Tat) and the RNA splicing regulator (Rev), which are necessary for viral replication (Cohen et al. 2010). The initial viral proteins to participate in the replication cycle are the Env proteins; gp120 and gp41 mediate the binding to the cell and the fusing to the cellular membrane, respectively (Arrildt et al. 2012). It is believed that infected monocytes act as "Trojan Horses" to pass the blood-brain barrier and allow HIV to enter the brain. The virus spreads throughout the brain when the monocytes develop into macrophages and create fresh HIV virions (Gaskill et al. 2017). Perivascular macrophages, microglia, and astrocytes are the main targets of HIV infection (Joseph et al. 2015). However, it is still unknown how astrocytes enter these cells and what part they play in HIV neuropathogenesis overall (Luo et al. 2015; Joseph et al. 2015). Numerous inflammatory host and viral factors, including as cytokines, chemokines, and viral proteins, are produced and secreted by infected cells, mainly macrophages, monocytes, and microglia. Chronic neuroinflammation is the outcome of these factors (Hong et al. 2015; Zayyad et

al. 2015). Antiretroviral treatment (ART) quickly lowers the plasma viral load to values below the detection limit when HIV-1 infection is treated (Palella et al. 1998). HIV eradication is hampered by the existence of a long-lasting reservoir of latently infected cells carrying proviral HIV-1 DNA, even while therapy with ART stops viral replication (Robb et al. 2016). After stopping ART, this reservoir—which was created early in the infection—continues to act as a source of viral reproduction. Peripheral $CD4^+$ T cell levels of HIV-1 DNA dramatically drop when ART is started during acute infection; those treated at the earliest stage of acute infection show the largest reduction (Ananworanich et al. 2016; Ananworanich et al. 2012). Advances in HIV therapy and prevention have been made possible by contemporary biotechnological techniques like gene editing, HIV vaccines, recombinant viral vectors, and enhanced drug delivery systems (Pantaleo et al. 2010). This review attempts to investigate these contemporary biotechnology methods, evaluate their present state, and look into their prospective applications in the future.

Biotechnological techniques in HIV management

Gene editing technology (CRISPR-cas System)

Gene-editing techniques that are related to the clustered regularly interspaced short palindromic repeat (CRISPR)-associated nuclease 9 (Cas9) have been created and marketed as efficient treatments for HIV-1 infection in recent years. However, new gene-targeting techniques that are based on or work similarly to CRISPR/Cas9, such as prime editing, base editor, SHERLOCK, DETECTR, PAC-MAN, ABACAS, and pfAGO, have been created and optimized for the identification of pathogens and the correction of disorders (Zhang et al. 2022).

Table. 1: Application of CRISPR-cas system

CRISPR-cas system	Delivery	Target	Cell type/ organisms	Animal Model	Ref.
SpCas9	Lentivirus, Adenovirus, Electroporation& Transfection,	LTR, Rev, Gag, Pol, Env, CCR5, CXCR4, Restriction factors,	HEK293T, 293T-CD4-CCR5, 293 Primary T cells, Hela, TZM-b1, Jurkat, SupT1, K562, hPSC, iPSC, , U937, JLat10.6, J.Lat FL, CEMss, C8166, Ghost, pMoHIV, CHO, human CD34+ HSPCs, CHME5	Mouse	Ebina et al. 2013; Hu et al. 2014; Liao et al. 2015; Wang et al. 2014; Wang et al. 2016; Lebbink et al. 2017; Cho et al. 2013; Li et al. 2015
SaCas9	AAV, Lentivirus & Transfection	TR, Gag, proviral HIV DNA, CCR5, CXCR4,	HEK293T, TZM-bl, C11, Ghost, Jurkat, primary CD4+ T, human CD34+ HSPCs, J-Lat 6.3	Tg26 transgenic mouse, SIV-infected macaques	Xiao et al. 2019a; Olson et al. 2020; Zhang et al. 2022
CRISPRa	Transfection, Lentivirus & Electroporation	LTR, APOBEC3G, APOBEC3B, T5, T6,	TZM-b1, J-Lat, CHME5 microglial cell, C11, HEK293T, ACH2, J1.1, CEM LChIT 3.2, Hela, U1	No	Zhang et al. 2022
CRISPRi	Lentivirus	PSMD 1, PSMD 3, PSMD 8, Spt4, Spt5, host factors (FTSJ3, TMEM178A, NICN1)	Jurkat 2D10	No	Krasnopolsky et al. 2021; Zhang et al. 2022)

CPF1	Adenovirus & Lentivirus	CCR5, LTR, Gag, Vpr, Tat, Rev	TZM-b1, SupT1-R5, Primary CD4+T cells, HEK293T	No	Zhang et al. 2022; Gao et al. 2020)

Even if the use of CRISPR/Cas gene editing to treat HIV-1 has advanced and improved, DSB still causes some unavoidable flaws in the NHEJ and HDR processes that could result in uncontrollable adverse effects. Alexis et al. created a unique genome editing method known as "base editor" to prevent DSB. It consists of a DNA-modifying enzyme, such cytidine deaminase, and a programmable DNA-binding protein, like catalytically impaired Cas9, which is incapable of cleaving DNA, or nickase Cas9 Cytosine base editor (CBE) is a "base editor" that can be effectively modified. Its "active window" is usually bases 4–8 of the protospacer, where PAM is counted as 21–23 bases. As a result, it can successfully fix a variety of point mutations linked to human illnesses (Komor et al. 2016). Base editors can successfully install four transition mutations (C→T, G→A, A→G, and T→C), but at this point, the remaining eight conversions (C→A, C→G, G→C, G→T, G→T, A→C, T→A, and T→G) cannot be generated. By combining reverse transcriptase with catalytically damaged Cas9 (H840A), a novel gene-editing method known as Prime Editing (PE) was created to address this issue. Using a primary editing guide RNA (pegRNA), which binds specifically to the target site and encodes for the necessary editing sequence, new genetic information is directly written into the designated DNA spot in the PE system (Anzalone et al. 2019).

HIV-1 genomic RNA and inbound RNA in infected cells can be targeted by Cas13a. For quick, easy, and sensitive target gene detection, specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) was proposed by combining the Cas13a enzymology of RNA cleavage with nucleic acid preamplification of isothermal recombinase polymerase or reverse transcription recombinase polymerase (Kellnar et al. 2019) (Gootenberg et al. 2017). Similar to SHERLOCK, the Cas12a derived DNA endonuclease-targeted CRISPR trans reporter (DETECTR) can cause DSB, which induces nonspecific

DNA cleavage (Li et al. 2019). Therefore, DETECTR can break the ssDNA reporter with fluorescence and quenching groups linked at either end. It may detect HIV-1 provirus DNA in AIDS patients using DETECTR with specific gRNA/Cas12 (Li et al. 2019). The prophylactic antiviral CRISPR approach in human cells (PAC-MAN), which is also used to manage HIV-1/AIDS, was created by Abbott et al. in response to the COVID-19 pandemic caused by SARS-CoV-2 in the previous year. It is based on Cas13d and targets SARS-CoV-2 and all sequenced coronavirus strains (Abbott et al. 2020). The antibody and CAS fusion (ABACAS) technique was created by fusing Cas13 with a particular antibody of the primary viral structural proteins, namely S protein, in order to target SARS-CoV-2 more precisely.Since HIV-1 can also enter target cells by endocytosis, combining ABACAS with Cas13 and an HIV-1 antibody may increase the virus's selectivity and RNA cleavage efficiency (Zhang et al. 2022).

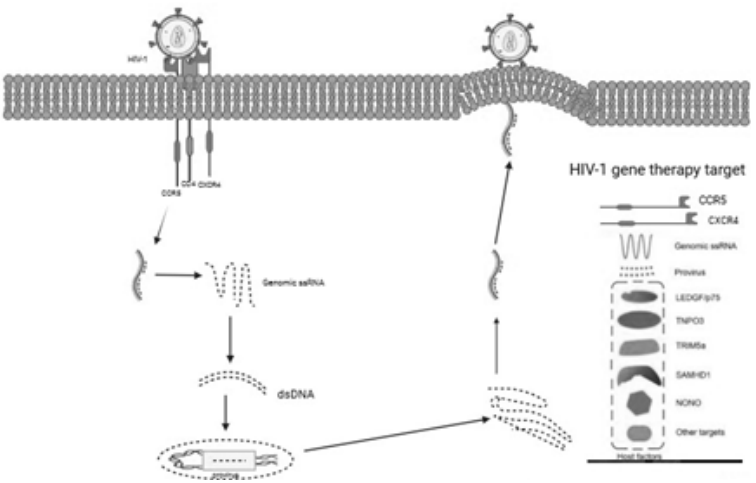

Fig. 1. Gene Editing Targets in the HIV-1 Life Cycle: HIV-1 gene therapy targets can be categorized into four main areas: **1. HIV-1 Co-Receptors**: Since HIV-1 invades immune cells via the CCR5 and CXCR4 co-receptors, gene-editing technologies can be employed to silence or mutate these co-

receptors, thereby rendering cells resistant to HIV-1 infection. **2. HIV-1 RNA Genome or Provirus**: CRISPR-Cas systems like Cas12a and Cas13a can target and degrade HIV-1 RNA. Additionally, proviral DNA can either be excised using gene-editing tools or reactivated through CRISPRa as part of the "shock and kill" approach. **3. Essential Host Factors**: Host proteins like LEDGF/p75 and TNPO3, which are crucial for the HIV-1 life cycle, can be disrupted through gene-editing strategies to inhibit viral replication within host cells. **4. HIV-1 Restriction Factors**: Gene-editing tools or CRISPRa can be used to upregulate restriction factors such as TRIM5α, SAMHD1, and NONO, or to create enhanced variants that bolster cellular defences against HIV-1 (Zhang et al. 2022).

RNA interference (rnai) therapies for HIV:

The endoribonuclease Dicer recognizes and cleaves intracellular long double-stranded RNA (dsRNA) intermediates or endogenous microRNAs (miRNA) into small interfering RNAs (siRNAs) of 21–23 nucleotides, which starts the conventional route of RNA interference (Ding et al. 2007). An essential part of the RNA-induced silencing complex (RISC) is the Argonaute protein, more especially Ago2 in mammals. After one of the siRNAs is loaded onto Ago2, RISC uses the loaded siRNA's guide strand to locate and cleave the complementary mRNA in the center of the siRNA:mRNA duplex. In the meantime, the loaded siRNA's non-targeted (passenger) strand is removed (Svoboda et al. 2020). Artificial miRNA can be manufactured and expressed intracellularly from a transgenic construct, or synthetic mature siRNA or short shRNA can be utilized to create artificial dcrRNA, which can be transfected into the cell (Zeng et al. 2002).

Anti-HIV^{-1}-SiRNAs:

Anti-HIV-1 siRNAs may have an advantage over existing treatments in that their sequences may be modified to specifically target a patient's unique viral strains, offering a more individualized therapeutic strategy. Additionally, they would create a number of new targets for HIV-1 combination medication therapy (Mizrahy et al. 2017). A major challenge for the development of anti-HIV-1 siRNAs

is that lymphocytes, which represent the major cell-type for HIV-1 replication, are widely distributed in the body and extremely difficult to penetrate with existing siRNA delivery technologies. Anti-HIV-1 siRNAs must be oral, have a long shelf life, and reduce viral loads to undetectable levels with at least one dose per day in order to compete with existing HIV-1 medication regimens (Scarborough et al. 2017).

Anti-HIV-1-ShRNAs

Three extensive siRNA screens have been carried out to find human genes involved in HIV-1 replication. Nevertheless, only three genes (MED7, MED8, and RELA) shared characteristics throughout the investigations, and none of them included well-known co-factors of HIV-1 replication like LEDGF/p75 (Brass et al. 2008; Zhou et al. 2008; Eekels et al. 2011). 30 human genes that have been linked to HIV-1 replication in the past, and various shRNAs target these genes (Eekels et al. 2011). They determined that TRBP, ALIX, and AGT6 were the most appropriate genes for long-term suppression of HIV-1 replication in shRNA-transduced T lymphocyte cell lines with low toxicity. CCR5 was not evaluated because it was already shown that it was a viable target for shRNA treatment. It would be extremely difficult for HIV-1 to become resistant to the reduction of a crucial component required for its reproduction if the mRNAs of cellular proteins essential in HIV-1 replication were targeted (Scarborough et al. 2017). Every coding and non-coding area of HIV-1 RNA can be targeted by shRNAs, and one shRNA that targets the overlapping tat/rev exon 1 reading frame has progressed to clinical trials. The fact that the target location of shRNAs targeting HIV-1 RNA is conserved across circulating HIV-1 strains is the most crucial factor in their design (Scarborough et al. 2017). Additionally, HIV-1 can evade shRNAs by acquiring mutations in sequences beyond the target site that affect its accessibility, most likely as a result of RNA structural alterations.RNA structural conservation may potentially be just as crucial for the creation of shRNAs as sequence conservation (Herrera-Carrillo et al. 2015; Westerhout et al. 2008).

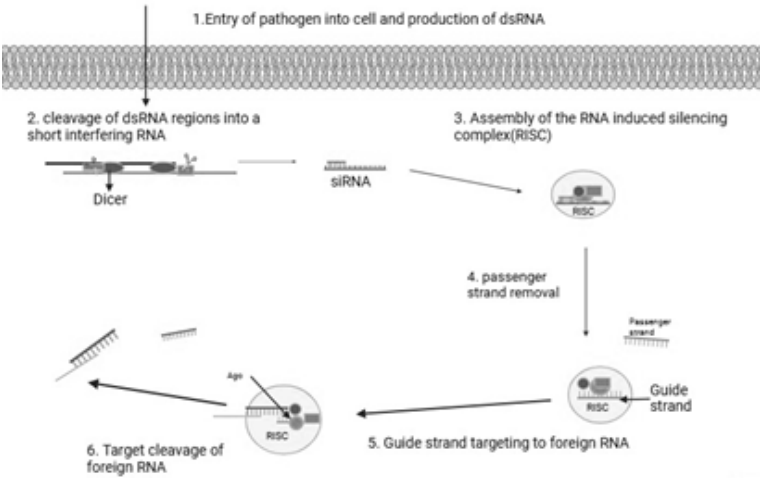

Fig. 2. Human micro-RNA (miRNA) pathway (Scarborough et al. 2017)

Monoclonal antibodies

Over the past few decades, a great deal of information has been gathered about monoclonal antibodies that target the HIV envelope (Env) glycoprotein. Both HIV-infected people with chronic progressive infection and people who control HIV without antiretroviral medication have been found to have a wide variety of antibodies with exceptional neutralizing potency and breadth against vast panels of cross-clade viruses (Freund et al. 2017; Walker et al. 2011). The CD4 binding site antibodies VRC01, 3BNC117, and VRC07–523LS; the V3-loop-directed antibodies PGT121 and 10–1074; the V1/V2 loop antibodies PGDM1400 and CAP256-VRC26.25; and the membrane-proximal region (MPER)-specific antibody 10E8 are among the monoclonal antibodies in clinical development for treatment and prevention (Doria-Rose et al. 2014; Sok et al. 2014). These antibodies target multiple epitopes on the surface of Env. In phase 1 and 2 studies, these antibodies—which were chosen for their capacity to bind and kill virions—have demonstrated encouraging outcomes by lowering plasma viremia and postponing viral rebound after

treatment interruptions for antiretroviral therapy (Rudicell et al. 2014; Rossignol et al. 2021). However, a broadly neutralizing monoclonal antibody's capacity to bind firmly to highly conserved areas of the viral envelope is a crucial factor in determining its capacity to induce neutralization (Lu et al. 2016a; Gautam et al. 2016). Rather, both Fab- and Fc-mediated processes are necessary for the removal of HIV reservoir cells. In order to eliminate the antibody-opsonized target, antibodies must recognize an infected cell and use Fc receptors (FcγRs) expressed on the cell to attract innate effector cells including neutrophils, monocytes, and natural killer cells (Ren et al. 2018; Rossignol et al. 2021). While earlier research has shown that broadly neutralizing antibodies (bNAb) may identify and eradicate infected cells, these antibodies only target neutralizing epitopes on the Env trimer, which could not fully represent the epitope landscape on the surface of the infected cell (Moore et al. 2006).

Vaccine development

Current strategies for preventing and treating HIV/AIDS include antiretroviral therapy (ART) and pre-exposure prophylaxis (PrEP), which have transformed AIDS into a manageable chronic condition. However, these treatments are costly, require strict adherence, may cause side effects, and face challenges like drug resistance. A prophylactic vaccine remains crucial to ending the epidemic, with recent advances in HIV immunogen design and mRNA vaccine technology offering promising developments (Mu et al. 2021). The basis of an mRNA vaccination is the mRNA encoding one or more immunogens, which are then translated into proteins when they are delivered into host cells.A possible new method of delivering antigens is using RNA-based vaccinations (Bloom et al. 2021).

Non-amplifying mRNA, encoding only the immunogen, offers a straightforward and cost-effective approach to mRNA vaccine development. Initially, such mRNAs were used alone or in liposomes as immunogen sources. Recent advancements, including codon optimization, 3′ and 5′ untranslated region modifications, nucleoside alterations, poly-A tail enhancements, and improved purification

methods, have significantly boosted their immunogenicity and safety (Petsch et al. 2012; Alameh et al. 2022).

Self-amplifying RNAs (saRNAs) are engineered replicons derived from RNA viruses that encode both the immunogen and replication machinery. Once inside the cell, saRNAs replicate, increasing immunogen production compared to non-amplifying RNAs. This allows saRNAs to achieve comparable immunogenicity at lower doses. They can be delivered directly or as viral replicon particles (VRPs), offering versatile approaches for vaccine development (Geall et al. 2013; Bloom et al. 2021).

Nanotechnology based drug delivery

A new and innovative approach to treating complicated illnesses is nanomedicine. With its high drug-loading capacity, ability to deliver medications to targeted diseased tissues, and increased biocompatibility, nanomedicine has numerous advantages over traditional medicine (Patra et al. 2018). Nanoparticles can also increase drug stability and control the release of the drug. Nanoparticles typically range from 1 to 100 nanometers. In addition, the composition of nanoparticles can be inorganic or organic. Nanoparticles can have a variety of shapes to meet the experiment's needs. Several nanomaterials, including dendrimers, liposomes, micelles, and nanosuspensions, have been studied for their potential application in HIV-1 infection (Fletcher et al. 2014).

Repeatedly branching molecules that radiate from a central core make up dendrimers. Their dynamic interior voids, restricted molecular weight distribution, and overall homogeneity make them very distinctive.By conjugating or absorbing pharmaceuticals onto their surface or by encapsulating them in their interior cavities, dendrimers can be utilized as nanocarriers (Dutta et al. 2007).

Micelles are lipid molecules that cluster spherically in aqueous solutions; pharmaceuticals can be encapsulated in the hydrophobic core of a micelle by forming a sort of shell around the water-soluble exterior (Chiappetta et al. 2010).

One or more phospholipid bilayer membranes around an aqueous center form the spherical structures known as liposomes. Liposomes offer many advantages as drug carriers owing to their nontoxic, non-immunogenic, biocompatible and biodegradable nature The transdermal delivery of Indinavir – a protease inhibitor – was significantly enhanced by use of ethanolic liposomes (Dubey et al. 2010).

Colloidal dispersions of medication particles that are nanosized and usually stabilized by a surfactant to prevent agglomeration are known as nanosuspensions. Cellulosics, lecithin, poloxamer, and polysorbates are popular stabilizers used in nanosuspensions. Drugs that are broken down or not absorbed in the GI tract (GIT) and/or have demonstrated only modest increases in bioavailability by stabilizing with surfactants are more suited for injectable nanosuspensions. Numerous antiretroviral nanosuspensions have produced prolonged release characteristics that could enable reduced dosage frequency and enhanced adherence (Roy et al. 2015).

Challenges and limitations

CRISPR-cas9

This is still a problem for HIV^{-1}/AIDS gene therapy in clinical research employing CRISPR/Cas9. In order to improve the practice in future applications, we should: (1) create more specific gRNA and Cas9 mutants to minimize off-target effects; (2) create single- or multi-functional base editors that can edit bases directly and accurately without DSB-induced NHEJ or HDR; (3) create Cas9 variants with flexible PAM sequences to increase the use of CRISPR/Cas9; (4) investigate new vehicles for the safe and effective delivery of gene-editing tools; and (5) optimize the HIV^{-1}/AIDS animal models and conduct more in vivo gene therapy experiments, which will set the groundwork for clinical use. With the aforementioned developments, genome editing technologies like CRISPR/Cas9 could lead to interesting and optimistic advancements in HIV^{-1}/AIDS gene therapy (Zhang et al. 2022).

RNAi

Gene therapy employing nucleotide-related drugs including ribozyme, antisense oligonucleotides, and siRNA has advanced significantly during the last 20 years. The therapeutic use of siRNA/shRNA in the treatment of HIV infections is, however, constrained by three factors. These three problems are the ineffectiveness of delivering nucleotide-derived medicines to target cells, the toxicities of RNA interference because of off-target effects, and the challenges of validating possible treatment leads from un vitro screening using an in vivo model (Zhang et al. 2007). One of the main barriers keeping RNAi from being useful therapeutic agents is cytotoxicity. By overloading the cellular RNA machinery, siRNA or shRNA can also have non-specific effects by blocking the normal function of endogenous miRNAs and blocking non-target mRNAs through sequence complementarity. The following proper design is essential to improve the specificity and reduce the toxicity of siRNA/shRNA: (i) reduce homology to non-target mRNAs; (ii) steer clear of perfect dsRNA stretches of 11 bp; and (iii) reduce inclusion of the sense strand into RISC (Boden et al. 2004).

Before RNA interference (RNAi) can be used as a viable treatment for HIV infection and other human diseases, a better knowledge of the mechanisms causing toxicity is necessary. Nowadays, conditional expression schemes are another way to reduce the toxicity of RNA interference, and doxycycline or ecdysone-controlled reversible on/off switch systems are already accessible over pol III and pol II promoters (Zhang et al. 2007; Wiznerowicz et al. 2006).

Vaccines & antibodies & nanotechnology

HIV vaccine research has largely aimed to generate antibodies that block the virus from infecting host cells, often using protein subunit vaccines with recombinant viral proteins as immunogens (Haynes et al. 2017). Challenges in developing broadly neutralizing antibody (bnAb) vaccines include tolerance mechanisms that limit bnAb B cell precursor development, peripheral anergy, and the unique traits required for bnAbs to neutralize HIV. Additionally, due

to HIV's rapid mutation and integration into host genes, achieving sterilizing immunity requires sustained, high levels of antibodies targeting multiple bnAb Env epitopes at the time of infection (Haynes et al. 2019). It has been gradually demonstrated that polyvalent HIV vaccines may be created and used to target conserved regions on the viral envelope, despite the high rate of variability (Marusic et al. 2009). Since most HIV strains can be neutralized by broadly neutralizing antibodies (bNAbs), this is the focus of the majority of current efforts. One significant benefit of bNAbs is their broad cross-reactivity, which allows them to neutralize a variety of HIV strains (Del Moral-Sánchez et al. 2019; Gruell et al. 2018).

However, by improving drug delivery, triggering efficient immune responses against HIV-infected cells, and—most importantly—directly targeting HIV latent reservoirs, nanomedicine-based techniques can enhance HIV therapy. Once medicines are combined with nanomedicine, there may be a chance to cure HIV. The clinical use of nanomedicine-based treatments has been sluggish, despite the field's revolutionary potential to advance anti-HIV medicine. To accelerate clinical translation and advance anti-HIV nanomedicine, more research is required to discover and better describe the pharmacokinetics, effectiveness, and biodistribution of nanotherapeutics (Andre et al. 2023).

Conclusion

Advancements in modern biotechnology have radically transformed HIV prevention, diagnosis, and treatment, presenting promising new methods for directly targeting the virus and overcoming longstanding challenges. Among these innovations, genome editing technologies, particularly CRISPR-Cas9, stand out for their ability to potentially modify the HIV genome and address the issue of latent reservoirs. Likewise, RNA interference (RNAi) has proven effective in silencing HIV-related genes, offering a precise method to hinder viral replication.

Monoclonal antibody therapies have also garnered attention for their ability to neutralize a wide range of HIV strains, providing a valuable addition to traditional antiretroviral

therapies. These treatments, often used in combination with existing drugs, have shown improved outcomes in controlling the virus and reducing viral loads. Concurrently, vaccine development has made significant strides, particularly with mRNA-based platforms and nanoparticle delivery systems, which have the potential to elicit stronger and more lasting immune responses, making long-term prevention and even immunity a tangible possibility.

On the diagnostic front, technological progress has enhanced the sensitivity, speed, and accessibility of HIV detection. From advanced point-of-care devices to sophisticated molecular assays, these innovations allow for earlier detection and continuous monitoring, contributing to better patient management. Furthermore, the integration of artificial intelligence and machine learning in biotechnological research is accelerating the discovery of novel therapeutic targets and optimizing treatment protocols.

However, challenges remain that must be addressed to fully realize the potential of these advances. HIV's genetic variability, its ability to establish latent reservoirs, and the complex dynamics between the virus and the immune system continue to complicate efforts toward a definitive cure. In addition, issues such as the high cost of new treatments and unequal access to healthcare pose significant barriers to the global impact of these innovations. Overcoming these obstacles will require sustained investment in research, international collaboration, and policies aimed at ensuring equitable access to new therapies and technologies.

In conclusion, modern biotechnological advances offer groundbreaking opportunities to reshape the future of HIV management. From genome editing and monoclonal antibody therapies to next-generation diagnostics and vaccines, these innovations are redefining how we approach the virus. While the journey toward eradication is still complex, the combination of scientific progress and global cooperation provides a clear path toward a world where HIV is no longer a significant threat to public health.

Future perspective

Continuous advancements in vaccine research, RNA treatments, and gene editing have the potential to completely transform HIV treatment. CRISPR-Cas9 and other gene-editing methods have the ability to target and eradicate the virus at the genetic level, while RNA-based treatments offer a more accurate method of regulating viral reproduction. mRNA vaccines' success against COVID-19 has raised hopes for comparable approaches to HIV prophylaxis. Machine learning and artificial intelligence are also speeding up medication research and making more individualized treatment plans possible. Addressing global disparities and moving closer to making HIV preventive, controllable, or even curable need ensuring that these discoveries are widely accessible.

Conflict of interest

There isn't any conflict of interest with this study.

Author contributions

Acquisition and interpretation of data is done by Tiyasha Saha., Tanisha Bhowick and Dipti Das. Conception, design and revising of the article are done by Rupesh Dutta Banik.

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The Toxicological Consequences of Naproxen

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ABSTRACT

Naproxen, a widely used nonsteroidal anti-inflammatory drug (NSAID), provides relief from various pain and inflammatory conditions, including arthritis, menstrual cramps, gout, and tendinitis. This article examines the mechanisms of action, diverse applications, and user experiences associated with naproxen. As a member of the propionic acid class, naproxen effectively inhibits the synthesis of prostaglandins, reducing inflammation and pain. The drug is available in various forms, including immediate-release, delayed-release, and extended-release tablets, as well as oral suspensions and capsules. Its over-the-counter availability has contributed to its popularity, particularly for those managing chronic pain. Despite its efficacy, naproxen may cause gastrointestinal disturbances and other side effects, particularly when used long-term. Safety concerns, such as the risk of gastrointestinal bleeding and cardiovascular issues, emphasize the importance of using the lowest effective dose for the shortest duration. This review highlights the importance of understanding naproxen's benefits, potential drawbacks, and safety precautions to guide informed decision-making in pain management.

Keywords: *Cardiovascular Risks, Drug, Gastrointestinal Toxicity, Naproxen, Toxicology*

Introduction

Millions of people rely on naproxen, a widely used nonsteroidal anti-inflammatory drug (NSAID), to relieve pain and inflammation. It is commonly used to manage conditions such as arthritis, menstrual cramps, gout, tendinitis, and bursitis(Nissen et al., 2016). Naproxen works by blocking the production of prostaglandins, which are hormone-like substances responsible for pain and inflammation(Davies & Anderson, 1997). By reducing these chemicals in the body, naproxen helps decrease swelling, stiffness, and overall discomfort.

This medication is available in different forms to meet various needs. Immediate-release tablets provide quick pain relief, while delayed-release and extended-release versions offer longer-lasting effects, making them ideal for chronic conditions like arthritis(Weisman & Brunton, 2020). Oral suspensions are available for individuals who have difficulty swallowing pills, and liquid-filled capsules provide another easy-to-ingest alternative. Over-the-counter naproxen, often sold under the brand name Aleve, is commonly used for mild to moderate pain, while prescription-strength versions such as Anaprox and Naprosyn are recommended for more severe conditions(Smith et al., 2017).

Naproxen's popularity is evident in its widespread use, with millions of prescriptions written annually in the United States. The accessibility of over-the-counter forms has further increased its usage, making it one of the most commonly purchased NSAIDs(Hui et al., 2017). Many users report significant relief from pain and inflammation, particularly those suffering from chronic conditions such as arthritis. Regular use often leads to improved mobility and reduced stiffness, allowing individuals to maintain a better quality of life.

However, like any medication, naproxen comes with potential side effects. Some individuals experience gastrointestinal issues such as nausea, heartburn, and stomach discomfort(Oliveira

et al., 2022). Others report headaches or dizziness as possible adverse effects. To minimize stomach-related issues, it is generally advised to take naproxen with food or milk. While many find the medication effective, responses can vary from person to person(Chaudhuri et al., 2013).

Safety should always be a priority when using naproxen. Those with pre-existing gastrointestinal or cardiovascular conditions should consult a healthcare professional before taking it. Long-term use of NSAIDs, including naproxen, may increase the risk of serious complications such as gastrointestinal bleeding, heart attacks, or strokes(Frost et al., 2014). To reduce these risks, medical professionals recommend using the lowest effective dose for the shortest possible duration(Moyer, 1986).

Overall, naproxen remains a highly effective and widely used option for managing pain and inflammation. Its versatility, availability, and effectiveness make it a staple in both over-the-counter and prescription medicine(Weisman & Brunton, 2020). While it provides significant relief for many individuals, understanding its uses, potential side effects, and safety precautions is essential for making informed healthcare decisions(Huntjens et al., 2006).

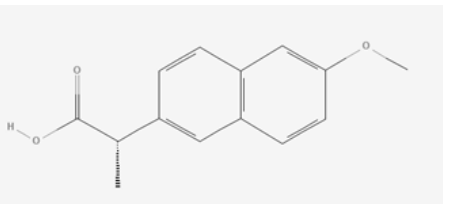

Fig 1: Structure of Naproxen (PubChem, https://pubchem.ncbi.nlm.nih.gov)

Naproxen Pharmacodynamics

Naproxen is a widely used non-steroidal anti-inflammatory drug (NSAID) known for its analgesic, anti-inflammatory, and antipyretic properties(Weisman & Brunton, 2020). Its primary mechanism of action involves inhibiting cyclooxygenase (COX) enzymes, specifically COX-1 and

COX-2. These enzymes play a crucial role in the production of prostaglandins, which are hormone-like substances responsible for triggering inflammation, pain, and fever by promoting vasodilation and increasing vascular permeability in affected tissues(Davies & Anderson, 1997).

When taken orally, naproxen is efficiently absorbed, reaching peak plasma concentrations within 2 to 4 hours in standard formulations and 1 to 2 hours in its more soluble form, naproxen sodium(Davies & Anderson, 1997). Due to its relatively long half-life of approximately 12 to 17 hours, it is often administered twice daily. The drug undergoes extensive hepatic metabolism, converting into inactive metabolites that are primarily excreted through the urine(Frost et al., 2014).

Naproxen is available in two formulations: free acid and sodium salt. Although both forms exhibit equivalent pharmacological and therapeutic effects, they differ slightly in absorption rates. At comparable doses—500 mg of naproxen being equivalent to 550 mg of naproxen sodium—the sodium salt formulation reaches peak plasma concentration about one hour after administration, whereas the free acid form takes approximately two hours(Moyer, 1986). This distinction is particularly important in acute pain management, where the faster absorption of naproxen sodium offers a quicker onset of relief(Davies & Anderson, 1997).

Pharmacokinetic Characteristics

Naproxen is characterized by rapid and complete absorption when administered orally or rectally. Different formulations, including immediate-release, enteric-coated, and controlled-release types, yield comparable peak plasma concentrations (Cmax), which typically range between 94 mcg/mL and 97.4 mcg/mL(Davies & Anderson, 1997).

A pharmacokinetic study revealed that 500 mg of immediate-release naproxen reaches peak concentration (Tmax) in approximately 3 hours, whereas the controlled-release formulation Naprelan (1000 mg) takes around 5 hours. Despite this variation in Tmax, the total drug exposure over 24 hours, measured by the area under the curve (AUC), remained nearly identical at 1446 mcg•hr/mL for the immediate-

release form and 1448 mcg•hr/mL for the controlled-release version(Moyer et al., 1986).

Another study comparing standard tablets to enteric-coated tablets found that the Tmax for Naprosyn was 1.9 hours, while for EC-Naprosyn, it was 4 hours. Corresponding AUC values were 767 mcg•hr/mL and 845 mcg•hr/mL, respectively. These findings highlight the importance of formulation selection in achieving optimal therapeutic outcomes (Davies & Anderson, 1997).

Factors Affecting Absorption

Food intake can slightly delay the absorption of naproxen when taken orally, but it does not significantly alter the overall amount of the drug absorbed. This means that naproxen can be taken with or without food without compromising its overall effectiveness.

Metabolism

Naproxen undergoes extensive liver metabolism through Phase I and Phase II processes. In Phase I, cytochrome P450 enzymes (CYP1A2, CYP2C8, CYP2C9) facilitate demethylation, converting naproxen into its active metabolite, 6-desmethylnaproxen [12]. Phase II metabolism involves glucuronidation by UGT enzymes (UGT1A1, UGT1A3, UGT1A6, UGT1A7, UGT1A9, UGT1A10, UGT2B7) and sulfation by sulfotransferases (SULT1A1, SULT1B1, SULT1E1), forming conjugated metabolites for excretion. (Oliveira et al., 2022)

Mechanism of Action

Naproxen works by inhibiting cyclooxygenase (COX) enzymes, reducing prostaglandin synthesis, which plays a key role in pain, fever, and inflammation. COX-1 supports essential functions like stomach lining protection and platelet aggregation, so its inhibition may cause gastrointestinal side effects(Nissen et al., 2016). COX-2, primarily involved in inflammation, is responsible for pain and swelling(Weisman & Brunton, 2020) . By non-selectively blocking both COX-1 and COX-2, naproxen effectively reduces pain and inflammation but carries a risk of gastrointestinal issues. It is well-absorbed,

with high bioavailability (~95%) and peak plasma levels occurring within 1–4 hours(Frost et al., 2014).

Excretion

Around 95% of naproxen and its metabolites are excreted in urine, mostly as conjugates, with minimal unchanged drug elimination. It is highly protein-bound (>99%), but at higher doses, saturation can lead to increased free drug levels(Moyer, 1986). The half-life of naproxen is 12–17 hours, with metabolite clearance affected in renal impairment(Oliveira et al., 2022).

In Vitro Toxicology Assay

- **Cytotoxicity Assessment:** The MTT assay evaluated naproxen's impact on cell viability at concentrations of 0.3–100 μM over 24, 48, and 72 hours. Results showed a time- and dose-dependent reduction in cell viability, with significant chondrotoxic effects observed in human chondrocytes (1–1,000 μM) ($p<0.01$.(Smith et al., 2017)
- **Enzyme Inhibition (COX-2 & 5-LOX):** Studies assessed how naproxen derivatives inhibit COX-2 and 5-LOX by determining IC50 values. MTT assays demonstrated a decline in optical density (OD), reflecting reduced cell viability (24-hour control OD: 0.369; 72-hour naproxen OD: 0.325). (Nedeljković et al., 2023)
- **Apoptosis Induction:** Annexin V-FITC tests indicated that prolonged exposure to higher naproxen concentrations triggered apoptosis. Naproxen-HBTA reduced melanoma cell proliferation (10–100 μM), inhibited migration and invasion, and suppressed colony formation. It also activated caspase-3, a key protein in programmed cell death, suggesting potential anticancer effects. (Ercolano et al., 2019)
- **Mutagenicity Potential:** Experiments on mammalian cells suggested naproxen may pose a genetic risk under specific conditions, emphasizing the need for caution with long-term exposure. (Huntjens et al.,2006) (Correia et al., 2014)

- **Toxicological Impact:** The primary adverse effects of naproxen stem from its role in prostaglandin suppression, leading to potential gastrointestinal issues and kidney damage, underscoring the importance of careful use. (Huntjens et al.,2006)

Conclusion

Naproxen is a commonly used NSAID known for its anti-inflammatory, pain-relieving, and fever-reducing effects. By inhibiting COX-1 and COX-2 enzymes, it effectively manages inflammation and pain but may also cause gastrointestinal and kidney-related side effects. It is well-absorbed, undergoes liver metabolism, and is mainly excreted through urine. In vitro studies indicate its cytotoxic effects, potential for genetic alterations, and ability to trigger apoptosis in specific cell types, suggesting possible anticancer applications. However, its long-term use should be approached with caution due to associated health risks. A thorough understanding of its pharmacological effects and safety considerations is essential for optimizing its therapeutic use while minimizing adverse outcomes.

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The Structure and Function of Surface Protein A in Bacterial Pathogenesis

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ABSTRACT

Surface Protein A (SPA) is a key surface-associated protein found in various pathogenic bacteria, including *Streptococcus pneumoniae*. SPA plays a critical role in the interaction between bacteria and host cells, facilitating adhesion, immune evasion, and biofilm formation, all of which contribute to bacterial virulence. Structurally, SPA proteins are characterized by their ability to bind to host immune components, such as immunoglobulins and complement proteins, which enable the bacteria to avoid immune detection and clearance. The protein's structure typically includes a series of repeating domains that are crucial for its interaction with host receptors. Additionally, SPA proteins are involved in bacterial aggregation and the formation of biofilms, providing the bacteria with a protective niche against environmental stressors, including antimicrobial agents and immune responses. This abstract reviews the structural features of SPA proteins and their functional implications in bacterial pathogenesis, highlighting their potential as therapeutic targets for the development of vaccines and novel antimicrobial strategies. Understanding the structure-

function relationship of SPA proteins is essential for designing interventions aimed at disrupting bacterial adherence and immune evasion, thereby mitigating infections caused by these pathogens.

Keywords: *Adhesion, Biofilm, Immune Evasion, Surface Protein A, Virulence*

Introduction

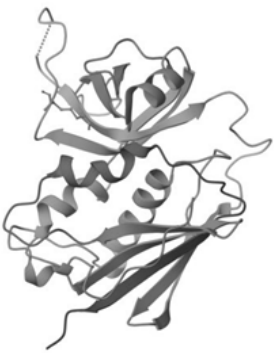
Staphylococcus aureus **is a major bacterial pathogen responsible for various infections, ranging from minor skin ailments to severe systemic diseases. One of its key virulence factors is Staphylococcal Protein A (SpA), (PDB ID:1SE2) a surface protein that plays a crucial role in bacterial survival by evading host immune responses. SpA binds to immunoglobulins, disrupts phagocytosis, induces inflammatory pathways, and contributes to biofilm formation, making it an essential component of** *S. aureus* **pathogenesis. This review explores the structure of SpA, its role in immune evasion, its contributions to different infections, and its potential as a target for novel therapeutic interventions. Additionally, it discusses SpA's evolutionary significance and challenges associated with targeting it for clinical applications.** *Staphylococcus aureus* **is a highly adaptable bacterial species capable of existing as a commensal organism on the human body while also acting as a significant pathogen. It is responsible for a wide range of infections, including skin infections, pneumonia, osteomyelitis, endocarditis, and sepsis. One of the primary reasons for its ability to cause such diverse infections is its extensive virulence factor repertoire, which includes toxins, adhesins, and immune-modulating proteins (Votintseva et al., 2011; O'Halloran et al., 2015).**

Among these virulence factors, Staphylococcal Protein A (SpA) is one of the most crucial. SpA enables *S. aureus* to evade the host immune system, allowing it to survive and persist in infected tissues. This protein is involved in various mechanisms, including immune system interference, inflammation induction, and biofilm development. Given its central role in bacterial pathogenesis, SpA is an attractive

target for therapeutic interventions aimed at reducing the severity of *S. aureus* infections.

Structural Features of Protein A

SpA is a **cell-wall-anchored protein** that is covalently attached to *S. aureus* via a specific sorting mechanism. It consists of **five immunoglobulin-binding domains**, labeled **E, D, A, B, and C**, which allow it to interact with host immune components. These domains are made up of **three-helix bundles**, a structural formation that enables strong binding to host proteins.



At the C-terminal region, SpA has a sorting signal that facilitates its attachment to the bacterial peptidoglycan layer. This ensures that SpA remains on the bacterial surface, where it can interact with host immune cells. However, *S. aureus* is also capable of releasing SpA into the extracellular environment, where it retains its immunomodulatory properties and affects immune responses even at a distance from bacterial cells.

Structural Features of Protein A

Domain Organization

Protein A (*SpA*) is composed of distinct structural components that contribute to its function in immune evasion (Swaminathan et al., 1995; Graille et al., 2000):

- **Immunoglobulin-binding domains:** It contains five homologous domains—E, D, A, B, and C—each approximately **58 amino acids in length**. These domains allow SpA to interact with host antibodies.
- **C-terminal sorting signal:** This region enables SpA to attach to the bacterial **peptidoglycan layer** with the help of **sortase enzymes**, securing it to the bacterial cell wall.
- **Flexible linker region:** This section provides structural adaptability, allowing **SpA to undergo conformational changes** when interacting with immune molecules.

Each of the **immunoglobulin-binding domains** is arranged in a **three-helix bundle** structure, a configuration essential for its ability to attach to host immune proteins.

Active Center and Key Amino Acids

The **active site** of Protein A is found within its immunoglobulin-binding domains and is composed of specific **amino acid residues** that contribute to its function:

- **Tyrosine (Tyr):** Plays a key role in **hydrophobic interactions** with the Fc region of **IgG antibodies**, enabling SpA to bind immunoglobulins and prevent immune recognition.
- **Leucine (Leu) and Phenylalanine (Phe):** Help **maintain the structural integrity** of the binding domains and contribute to **high-affinity interactions** with host immunoglobulins.
- **Glutamic acid (Glu) and Aspartic acid (Asp):** Involved in **electrostatic interactions** with host proteins, enhancing the stability of **SpA-host interactions**.
- **Lysine (Lys):** Crucial for **binding specificity**, particularly in interactions with **B cell receptors** and **tumor necrosis factor receptor-1 (TNFR-1)**, influencing immune modulation and inflammatory

responses.

The combination of **hydrophobic** and **charged amino acids** in these domains allows **SpA to effectively engage with immune system components**, thereby **interfering with immune signaling pathways** and promoting bacterial survival.

Immune Evasion Mechanisms of Protein A

1. Binding to Immunoglobulins

SpA has a unique ability to bind the Fc region of IgG antibodies, preventing opsonization and subsequent recognition by phagocytes. Opsonization is a key immune mechanism in which antibodies mark pathogens for destruction by immune cells such as neutrophils and macrophages. By interfering with this process, SpA allows *S. aureus* to remain undetected and persist in host tissues.

2. Disruption of B Cell Function

In addition to binding IgG, SpA can also interact with the Fab region of VH3 family B cell receptors, leading to non-specific B cell activation. This interaction ultimately results in B cell apoptosis, weakening the host's adaptive immune response and reducing its ability to generate effective antibodies against the infection. This mechanism provides *S. aureus* with an advantage by limiting the host's capacity to mount a long-term immune defense.

3. Activation of Inflammatory Pathways

SpA is capable of binding tumor necrosis factor receptor-1 (TNFR-1) on epithelial and immune cells. This interaction stimulates the production of pro-inflammatory cytokines, leading to excessive inflammation. While inflammation is an essential component of the immune response, uncontrolled inflammation can damage host tissues and facilitate bacterial survival. This mechanism is particularly relevant in conditions such as staphylococcal pneumonia, where SpA-induced inflammation exacerbates disease severity.

Role of Protein A in Various Infections

The role of SpA in diverse infections are mentioned below (Becker et al., 2014; Bear et al., 2023)

1. Skin and Soft Tissue Infections

SpA contributes to abscess formation, cellulitis, and impetigo by impairing phagocytosis and enhancing bacterial adherence to host tissues. It also aids in immune evasion, allowing *S. aureus* to persist in the skin and subcutaneous tissues.

2. Bloodstream Infections and Sepsis

In bacteremia and sepsis, SpA prevents immune clearance by interfering with opsonization and phagocytosis. Additionally, by stimulating TNFR-1, SpA promotes systemic inflammation, contributing to septic shock and organ dysfunction.

3. Respiratory Infections

SpA plays a key role in staphylococcal pneumonia by triggering excessive inflammation in lung tissues. This results in tissue damage and fluid accumulation, worsening respiratory distress in affected individuals.

4. Bone and Joint Infections

In **osteomyelitis and septic arthritis**, SpA promotes **bacterial attachment to osteoblasts** and contributes to **biofilm formation on bone surfaces**. These biofilms make infections harder to treat, leading to **chronic infections and increased antibiotic resistance**.

Evolutionary Significance of Protein A

SpA's widespread presence in **clinical isolates of *S. aureus*** suggests that it provides a strong **evolutionary advantage** for bacterial survival. The ability to bind immunoglobulins and modulate host immunity has likely contributed to *S. aureus* becoming one of the most successful **opportunistic pathogens** in human history.

Therapeutic Approaches Targeting Protein A

The therapeutic approach of SpA is mentioned below (Fox et al., 2021):

1. Vaccine Development

Because of its central role in immune evasion, SpA has been explored as a **vaccine target**. Some experimental vaccines aim to **generate antibodies that neutralize SpA**, preventing it from interfering with the immune response and allowing the body to effectively clear *S. aureus* infections.

2. Monoclonal Antibodies

Therapeutic **monoclonal antibodies** against SpA have been developed to **block its interaction with IgG**. By doing so, these antibodies restore **normal immune functions**, helping the host fight off infections more efficiently.

3. Small Molecule Inhibitors

Another promising approach involves **small molecule inhibitors** that disrupt SpA's ability to bind **immunoglobulins and TNFR-1**. These inhibitors could potentially **reduce bacterial virulence** and improve the effectiveness of conventional antibiotics.

Challenges in Targeting Protein A

Despite its potential as a therapeutic target, SpA presents several challenges:

- **Genetic variability**: Different *S. aureus* strains may have variations in SpA, affecting vaccine efficacy.
- **Functional redundancy**: *S. aureus* possesses multiple immune evasion strategies, meaning SpA inhibition alone may not completely neutralize its virulence.
- **Host interactions**: Because SpA interacts with **host proteins**, there is a risk of unintended immune system effects when targeting it therapeutically.

Conclusion

Staphylococcal Protein A is a key virulence factor that enhances *S. aureus* immune evasion, promotes persistent infections, and contributes to disease progression. Its diverse mechanisms of phagocytosis inhibition, B cell manipulation, and inflammation induction make it a major player in bacterial

pathogenesis. Given its crucial role, therapeutic interventions targeting SpA, including vaccines, monoclonal antibodies, and small molecule inhibitors, hold significant potential for treating *S. aureus* infections. However, further research is needed to overcome challenges related to bacterial resistance and immune system interactions.

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Understanding the Role of Lipid Biomarkers in Heart Failure and Cardiovascular Risk

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Abstract

Heart failure (HF) is a complex clinical syndrome influenced by various lipid biomarkers that contribute to disease progression, risk assessment, and prognosis. This review explores the role of lipid-derived biomarkers in heart failure and cardiovascular diseases, including total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides, lipoprotein(a) [Lp(a)], and other emerging markers. These biomarkers originate from lipid metabolism, inflammatory pathways, and endothelial dysfunction, influencing cardiovascular morbidity and mortality. Elevated LDL and small dense LDL particles significantly increase atherosclerotic risks, while impaired HDL function exacerbates inflammation and oxidative stress. Additionally, novel biomarkers such as circulating microRNAs, sphingomyelin phosphodiesterase 1, and endothelial dysfunction markers provide deeper insights into disease mechanisms and potential therapeutic targets. Understanding the pathophysiological roles of lipid biomarkers in heart failure can enhance early detection, risk stratification, and personalized treatment strategies, ultimately improving cardiovascular outcomes.

Keywords: *Atherosclerosis, Biomarkers, Cardiovascular disease, Dyslipidemia, Heart failure.*

Introduction Lipid metabolism and its dysregulation have long been implicated in the pathophysiology of heart attacks. Various lipid biomarkers provide critical insights into cardiovascular health by reflecting systemic inflammation, endothelial dysfunction, and atherosclerotic burden (Anderson et al., 2018). Understanding these biomarkers is vital for early detection and improved therapeutic strategies in cardiovascular disease management (Smith et al., 2018). Recent studies have highlighted the role of lipidomics in identifying novel biomarkers and developing targeted therapies (Harris et al., 2018; Taylor et al., 2018). This review aims to provide a comprehensive update on key lipid biomarkers, their clinical significance, and advancements in cardiovascular diagnostics.

Key Lipid Biomarkers and Their Role in Heart Attacks

Lipid biomarkers play a fundamental role in assessing cardiovascular risk, enabling healthcare professionals to predict and manage heart attack risks more effectively (Williams et al., 2018). These biomarkers serve as indicators of lipid metabolism, inflammation, endothelial dysfunction, oxidative stress, and lipid transport mechanisms (Brown et al., 2018). Several studies highlight their significance in both the early detection and long-term management of heart disease (Richards et al., 2018).

Low-Density Lipoprotein Cholesterol (LDL-C)

LDL cholesterol, commonly referred to as "bad cholesterol," is a key contributor to plaque formation in the arteries, which can lead to heart attacks (Jones & Miller, 2018). High LDL-C levels are directly associated with a greater risk of atherosclerosis, a condition where fatty deposits accumulate in blood vessels. To mitigate this risk, medical guidelines emphasize reducing LDL-C through lifestyle changes and cholesterol-lowering medications, such as statins and PCSK9 inhibitors (Smith et al., 2018).

High-Density Lipoprotein Cholesterol (HDL-C)

HDL cholesterol, often called "good cholesterol," is essential for maintaining cardiovascular health. It aids in the removal

of excess cholesterol from the bloodstream and transports it to the liver for excretion (Taylor et al., 2018). A deficiency in HDL-C has been linked to an increased likelihood of heart disease due to impaired cholesterol clearance. However, recent research suggests that the effectiveness of HDL function is more critical than its quantity in predicting cardiovascular risk (Harris et al., 2018).

Triglycerides (TG)

Triglycerides, a type of fat found in the blood, have been associated with cardiovascular disease, particularly in individuals with elevated LDL-C and low HDL-C levels (Lee et al., 2018). High triglyceride levels contribute to the formation of smaller, denser LDL particles, which are more likely to cause arterial blockages. Therapies targeting triglyceride reduction, including omega-3 fatty acids and fibrates, are increasingly being explored for their potential to improve heart health, especially in people with metabolic disorders (Brown et al., 2018).

Lipoprotein(a) [Lp(a)]

Lipoprotein(a) is a lesser-known but significant biomarker in cardiovascular health. Elevated levels of Lp(a) have been found to increase the risk of heart attacks due to their involvement in promoting inflammation, thrombosis, and endothelial dysfunction (Anderson et al., 2018). Unlike LDL-C, which can be influenced by diet and lifestyle, Lp(a) levels are largely determined by genetic factors, making it a challenging target for traditional interventions (Wilson et al., 2018). However, recent advances in genetic and lipid research are exploring targeted therapies to manage elevated Lp(a) levels.

Myeloperoxidase (MPO)

Myeloperoxidase is an enzyme released by white blood cells during inflammation. It contributes to oxidative stress and modifies LDL cholesterol, making it more likely to cause damage to the arterial walls (Williams et al., 2018). Increased MPO levels have been identified as a predictor of cardiovascular disease, as they contribute to plaque formation

and arterial stiffening. Because of this, MPO is increasingly being recognized as a valuable biomarker for early detection and risk assessment of heart attacks (Richards et al., 2018).

Circulating MicroRNAs (miRNAs)

MicroRNAs are small molecules that regulate gene expression and have recently been identified as potential biomarkers for cardiovascular diseases (Wilson et al., 2018). Specific miRNAs, such as miR-1, miR-133, and miR-208, have been associated with heart tissue damage and remodeling after a heart attack. Because they remain stable in the bloodstream and can be easily detected, they are considered promising tools for early diagnosis and personalized treatment approaches (Taylor et al., 2018).

Emerging Biomarkers in Cardiovascular Risk Assessment

Advancements in the study of lipids and metabolites have led to the discovery of new biomarkers, such as ceramides, sphingolipids, and oxidized phospholipids, which play a role in inflammation and the development of atherosclerosis (Harris et al., 2018). The integration of machine learning with lipid biomarker data is revolutionizing cardiovascular risk prediction by allowing more precise assessments and personalized treatments (Brown et al., 2018).

Table 1: Biomarkers involved in Heart Attack

Serial Number	Name of Biomarker (Lipid)	Origin of Biomarker	Role in Heart Attack
1	Total cholesterol, LDL cholesterol	Lipid metabolism in the liver and dietary intake	Premature cardiovascular morbidity and mortality (Homozygous familial hypercholesterolemia) (Brown et al., 2018; Wilson et al., 2018)
2	LDL cholesterol and small dense LDL particles	Metabolism of very-low-density lipoproteins (VLDL) produced by the liver	High CVD risks (Hypercholesterolemia) (Jones & Miller, 2018)

3	HDL cholesterol, Myeloperoxidase (MPO)	Dysfunction in reverse cholesterol transport; MPO from neutrophils and macrophages	Coronary heart disease (CHD), monocytosis, diabetes, hypertension, chronic kidney disease (Williams et al., 2018; Anderson et al., 2018)
4	TC, TG, HDL, LDL	Synthesized in the liver (endogenous) and absorbed from dietary sources (exogenous)	CVD and mortality (Dyslipidemia) (Smith et al., 2018; Harris et al., 2018)
5	LDL cholesterol	Metabolism of VLDL in the liver	Atherosclerotic peripheral arterial disease (Lee et al., 2018; Taylor et al., 2018)
6	Lipids, cholesterol, calcium, cellular debris	Liver synthesis, mevalonate pathway	Endothelial dysfunction, ischemic heart disease (IHD) (Taylor et al., 2018; Richards et al., 2018)
7	Myocardial remodeling markers	Heart muscle changes due to stress or injury	Diastolic dysfunction, heart failure (Richards et al., 2018)

Emerging Biomarkers and Future Perspectives

Recent research suggests that novel lipid biomarkers, including amino acids such as tyrosine, phenylalanine, and isoleucine, may offer valuable insights into cardiovascular risk (Lee et al., 2018). Advances in lipidomics and metabolomics present promising avenues for personalized medicine (Harris et al., 2018). These technologies enable better cardiovascular risk stratification and more precise therapeutic targeting (Taylor et al., 2018; Wilson et al., 2018).

Machine learning integration with lipid biomarker data has emerged as a revolutionary tool for enhancing cardiovascular risk prediction (Brown et al., 2018). Studies indicate that AI-driven lipidomics may refine diagnostic capabilities and treatment planning, leading to improved patient outcomes (Smith et al., 2018).

Conclusion

Lipid biomarkers serve as crucial indicators for diagnosing and managing heart attacks. Their roles in lipid metabolism, inflammation, and endothelial dysfunction highlight their significance in cardiovascular risk assessment (Richards et al., 2018). The inclusion of recent research strengthens the understanding of these biomarkers, underscoring their potential in clinical practice. Continued research on emerging biomarkers and novel lipidomic approaches is necessary to enhance early detection and therapeutic interventions for CVD.

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Structural and Functional Characterization of Pili P Protein in *Escherichia coli*

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ABSTRACT

Pili P, a type of pili found on the surface of *Escherichia coli*, plays a crucial role in the bacterial adherence to host tissues, contributing to the pathogenesis of various infections. This review aims to explore the structure, function, and role of Pili P in *E. coli*, highlighting its importance in bacterial colonization and virulence. Pili P are composed of protein subunits that assemble into hair-like structures, which facilitate adherence to epithelial cells and the formation of biofilms. This interaction is essential for the survival and persistence of *E. coli* in hostile environments, including the urinary tract, where it can lead to urinary tract infections (UTIs). The structure of Pili P involves an intricate arrangement of pilin subunits, with adhesins at the tip that mediate specific binding to host cell receptors. Additionally, the role of pili in the process of bacterial invasion, immune evasion, and resistance to host defense mechanisms is discussed. The review also covers recent advancements in understanding the molecular interactions between Pili P and host cells, as well as ongoing research aimed at targeting Pili P as a potential therapeutic strategy for treating *E. coli*-related infections. Understanding the structural dynamics and function of Pili P in *E. coli* can

provide valuable insights for developing new antimicrobial agents and treatment strategies.

Keywords: *Adhesion, Biofilm, E. coli, Pili P Protein, Virulence*

Introduction

Urinary tract infections (UTIs) are among the most prevalent bacterial infections, with *Escherichia coli* being the primary causative agent. The pathogenicity of uropathogenic *E. coli* (UPEC) is largely attributed to its ability to express surface appendages known as P pili, also referred to as pyelonephritis-associated pili. These filamentous structures facilitate bacterial adhesion to host tissues, playing a crucial role in the colonization of the urinary tract and the establishment of infection.

This review provides an in-depth examination of the structure, assembly, and functional roles of P pili, emphasizing their contribution to bacterial adhesion and immune evasion. Additionally, the active site and key amino acid residues involved in host receptor interactions will be discussed in detail, highlighting their significance in *E. coli* pathogenesis (Sheikh et al., 2017).

Structural Features of P Pili

P pili (PDB ID: 6NRV) are hair-like structures extending from the bacterial cell surface, measuring approximately 1–2 micrometers in length. These pili are composed of multiple protein subunits arranged in distinct structural domains: the pilus rod and the pilus tip (Hahn et al., 2001; Zheng et al., 2019).

Pilus Rod

The pilus rod serves as the primary backbone of P pili and is primarily composed of the major structural protein PapA. Around 1,000 PapA subunits polymerize in a right-handed helical configuration, providing the pilus with the mechanical strength necessary to withstand the shear forces present in the urinary tract. This helical arrangement also ensures the stability of the pilus during adhesion to host cells.

Pilus Tip

The distal end of the pilus rod is capped by the pilus tip, a flexible and specialized region essential for host recognition and adhesion. This region consists of several minor pilus proteins, each playing a specific role:

• **PapK:** Functions as an adaptor, linking the pilus rod to the tip fibrillum.

• **PapE and PapF:** Form a fibrillar structure that extends outward, positioning the adhesin for optimal receptor binding.

• **PapG:** The terminal adhesin that mediates bacterial attachment to host epithelial cells.

The spatial organization of these proteins ensures that PapG is optimally positioned for host cell recognition, enabling *E. coli* to establish infections in the urinary tract.

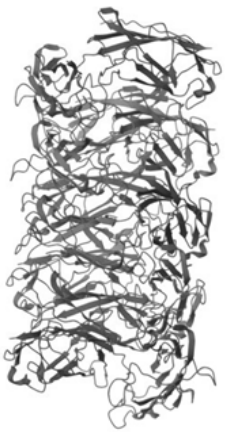

Fig 1: Structure of Pili (Zheng et al., 2019)

Chaperone-Usher Pathway: Assembly Mechanism

The biosynthesis of P pili is directed by the chaperone-usher pathway, a well-conserved mechanism among Gram-negative bacteria. This pathway ensures the proper folding, stabilization, and ordered assembly of pilus subunits

(Werneburg et al., 2018).

Periplasmic Chaperone (PapD)

Once synthesized in the cytoplasm, pilus subunits are translocated into the periplasmic space, where they interact with the chaperone protein PapD. PapD plays a dual role (Sarowar et al., 2016):

- Stabilization: It prevents the premature aggregation of pilus subunits by capping their interactive surfaces.
- Folding Assistance: It facilitates the proper conformational arrangement of subunits, ensuring they are correctly oriented for subsequent assembly.

PapD-subunit interactions are characterized by a donor strand complementation mechanism, where PapD contributes a beta-strand to complete the immunoglobulin-like fold of the subunit, ensuring structural stability.

Outer Membrane Usher (PapC)

The PapD-subunit complexes are transported to the outer membrane usher protein, **PapC**, which facilitates the stepwise polymerization of pilus components and their translocation to the bacterial surface. Key features of PapC include:

- **Subunit Polymerization:** Ensures the correct assembly of pilus proteins in a sequential manner.
- **Channel Formation:** Forms a transmembrane channel that allows for the extrusion of the growing pilus structure.

Through the concerted action of PapD and PapC, P pili are efficiently assembled, ensuring that the PapG adhesin is positioned at the pilus tip for effective host cell interaction.

Active Site and Key Amino Acids of PapG Adhesin

The PapG adhesin is a crucial determinant of UPEC virulence, as it mediates bacterial attachment to host cells. The active site of PapG is specifically adapted to recognize and bind to globoside receptors, particularly globotriaosylceramide (Gb3) and globotetraosylceramide (Gb4), which are highly

expressed on kidney epithelial cells (Zheng et al., 2019).

Receptor Binding Specificity

The binding specificity of PapG is critical for the tissue tropism of UPEC. Its ability to recognize Gb3 and Gb4 glycolipids enables it to target renal epithelial cells, contributing to the development of pyelonephritis.

Structural Composition of the Binding Pocket

Structural analyses of PapG have revealed a well-defined binding pocket designed to accommodate the carbohydrate moieties of host receptors. This pocket is stabilized by a network of hydrogen bonds and hydrophobic interactions, allowing for a strong and specific attachment.

Essential Amino Acid Residues in PapG

Several amino acids within PapG have been identified as crucial for receptor binding and adhesion:

- **Tyrosine (Tyr):** Forms hydrogen bonds with the galactose residues of host glycolipids, securing the receptor in place.
- **Phenylalanine (Phe) and Leucine (Leu):** Contribute to **hydrophobic interactions**, strengthening the adhesin-receptor complex.
- **Aspartic Acid (Asp) and Glutamic Acid (Glu):** Facilitate **electrostatic interactions**, enhancing binding affinity.

Mutagenesis studies have demonstrated that alterations in these key residues significantly diminish or abolish PapG's binding ability, highlighting their importance in UPEC adhesion.

Functional Role of P Pili in UPEC Pathogenesis

P pili play a crucial role in *E. coli* pathogenesis by promoting bacterial adherence and survival in the urinary tract.

Host Tissue Colonization

The PapG adhesin enables UPEC to attach firmly to kidney

epithelial cells, preventing bacterial clearance by urine flow. This adhesion is a key step in the establishment of UTIs, leading to bacterial invasion and inflammation.

Immune Evasion Strategies

UPEC employs several strategies to evade host immune defenses, including:

- **Mechanical Resilience:** The **helical structure** of the pilus rod allows UPEC to withstand urinary shear forces.
- **Antigenic Variation:** Variability in pilus components, including PapG, enables UPEC to evade immune recognition.

These adaptations contribute to the persistence of *E. coli* infections and increase the likelihood of recurrent UTIs.

Therapeutic Approaches Targeting P Pili

Given their pivotal role in UPEC pathogenesis, P pili have been explored as potential targets for anti-adhesive therapies aimed at preventing bacterial colonization (Duménil, 2019).

Pilus-Targeting Vaccines

Experimental vaccines have been developed to elicit immune responses against pilus subunits, particularly PapG, to block bacterial adhesion.

Small Molecule Inhibitors

Compounds that disrupt the interaction between PapG and host receptors are being investigated as potential therapeutic agents to prevent UPEC colonization.

Monoclonal Antibodies

Monoclonal antibodies directed against PapG could neutralize its adhesin activity, reducing bacterial attachment and enhancing clearance by the immune system.

Conclusion

P pili are essential virulence factors in UPEC, enabling bacterial adhesion and colonization of the urinary tract.

The structural organization of P pili, particularly the PapG adhesin, plays a critical role in host-pathogen interactions. By understanding the active site and key amino acids involved in receptor binding, novel therapeutic approaches targeting bacterial adhesion can be developed to mitigate UPEC infections. Future research should focus on refining vaccine formulations, small-molecule inhibitors, and monoclonal antibody therapies to effectively combat drug-resistant UPEC strains and reduce the burden of UTIs.

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Examining the bioactive properties of Cinnamonaldehyde and its Related Application

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ABSTRACT

The worldwide incidence of resistant to multiple medications bacterial infections keeps on rising. Antibiotic susceptibility to the antibiotics that are now accessible is growing as a worldwide health problem, triggering the swift creation of complementary antibacterial strategies. There has been a reaffirmed fascination with crafting antibacterial agents from natural sources, and transcinnamaldehyde is one naturally occurring compound that has received a lot of attention in recent years. Trans-Cinnamaldehyde has been shown to have significant antimicrobial activity, as well as a variety of other medicinal properties, making it an intriguing hit compound from which a number of derivatives have been developed. In some cases, these derivatives have been shown to be more active than both trans-cinnamaldehyde and commonly used antibiotics.As consequently, grasping the disinfecting tools of action yielded by such compounds is critical for permitting their development alongside the development of new antibacterial agents that can take advantage of similar mechanisms. The objective of this article is to give a brief insight into the latest information on the antibacterial qualities and equipment that operate of cinnamaldehyde

and its byproducts, and additionally to highlight significant contributions to this field of study. It is intended that the outcomes put forth in this work will assist in the advancement of fresh antibiotics.

Keywords: *Antibacterial, Cinnamaldehyde, Derivatives, Medicinal Properties, Resistance*

Introduction

Microorganisms, including fungi and bacteria, are omnipresent in nature. While many are harmless, certain pathogenic species cause infections that range from mild to life-threatening, especially in immunocompromised individuals. The global rise in conditions such as cancer, acquired immunodeficiency syndrome (AIDS), and autoimmune disorders has led to an increase in opportunistic infections, making the search for effective antimicrobial agents more critical (Havlickova, Czaika, & Friedrich, 2008).

Bacterial infections remain a significant challenge, particularly with the emergence of drug-resistant strains such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. These pathogens contribute to various health complications, including respiratory infections, sepsis, and wound infections. Due to the growing resistance of these bacteria to standard treatments, researchers are increasingly investigating plant-derived bioactive compounds with antimicrobial properties. Among these, cinnamaldehyde—a key constituent of cinnamon essential oil—has demonstrated promising antibacterial and antifungal activity (Friedman, 2017).

Cinnamaldehyde is a naturally occurring compound known for its distinct aromatic and flavoring properties. It has been widely used in the food and cosmetics industries and has recently garnered attention for its medicinal benefits. Studies indicate that cinnamaldehyde exerts antimicrobial effects by disrupting ATPase activity, interfering with cell wall synthesis, and modifying membrane integrity (Gill & Holley, 2004). Given its potential, further research into cinnamaldehyde's mechanisms of action and therapeutic applications is warranted.

Cinnamon: Primary Source of Cinnamaldehyde
Cinnamon is obtained from the bark of trees belonging to the *Cinnamomum* genus, native to regions of South Asia. These trees, particularly *Cinnamomum verum* and *Cinnamomum cassia*, are valued for their essential oils, which contain significant amounts of cinnamaldehyde. The unique aroma of cinnamon is attributed to volatile compounds present in the bark (Ooi et al., 2006).

The *Cinnamomum* genus comprises over 250 species distributed across various geographical locations. While *Cinnamomum cassia* is widely used in commercial spice production, *Cinnamomum verum* is recognized for its superior quality and higher medicinal value. The therapeutic properties of cinnamon extend beyond its flavoring applications, making it an important natural resource for pharmacological research (Rao & Gan, 2014).

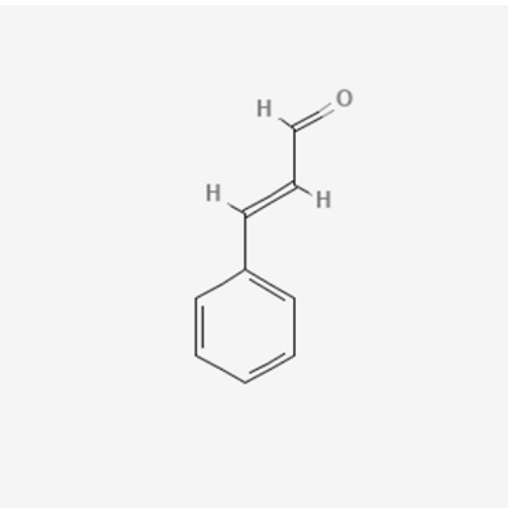

Fig 1: Structure of Cinnamaldehyde (PubChem)

Medicinal Properties of Cinnamaldehyde

Cinnamaldehyde has been extensively studied for its diverse medicinal benefits. It has demonstrated significant antimicrobial, antifungal, antioxidant, and anti-inflammatory

properties, making it a valuable candidate for pharmaceutical applications. Its ability to combat bacterial and fungal infections highlights its potential as a natural antimicrobial agent (Chang, Chen, & Chang, 2001).

Cinnamaldehyde as an Anti-Inflammatory Agent

Cinnamaldehyde and its derivatives have been shown to play a role in modulating inflammatory responses. The NF-κB signaling pathway, which regulates immune responses and inflammation, has been identified as a key target of cinnamaldehyde. Research indicates that cinnamaldehyde can suppress NF-κB activation and inhibit the production of inflammatory mediators such as prostaglandin E2 (PGE2). Additionally, it has been found to reduce cyclooxygenase-2 (COX-2) activity, further supporting its role in managing inflammatory conditions (Guo et al., 2006).

Synergistic Effects of Cinnamaldehyde with Antimicrobial Agents

Combination therapy has become an important approach in overcoming antibiotic resistance. Studies have explored the synergistic interactions between cinnamaldehyde and existing antifungal and antibacterial agents. For example, cinnamaldehyde has been shown to enhance the effectiveness of fluconazole against resistant fungal strains by increasing membrane permeability and facilitating drug uptake. Similar synergistic effects have been observed when cinnamaldehyde is used in combination with antibiotics such as amoxicillin and tetracycline. These findings suggest that cinnamaldehyde could be incorporated into combination therapies to improve antimicrobial efficacy (Gill & Holley, 2006).

Antimicrobial Activity of Cinnamaldehyde

Cinnamaldehyde, a major component of cinnamon essential oil, has gained significant attention for its ability to combat various microbial infections. Its antimicrobial properties stem from multiple mechanisms, including disruption of bacterial membranes, inhibition of biofilm formation, and interference with essential metabolic pathways (Szweda et al., 2021). Research has shown that cinnamaldehyde effectively inhibits

pathogens such as *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* by destabilizing their membrane integrity and reducing their ability to form protective biofilms (Oliveira et al., 2022).

In addition to its standalone antimicrobial effects, cinnamaldehyde has demonstrated synergy when used alongside conventional antibiotics. This combination enhances the efficacy of antibiotics while reducing the required dosage, which is particularly beneficial in managing multidrug-resistant bacterial infections. Notably, cinnamaldehyde has been reported to enhance the activity of antibiotics against resistant strains, including *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Enterobacter* species (Silva et al., 2022). This suggests that incorporating cinnamaldehyde into treatment strategies could help overcome antibiotic resistance by increasing bacterial susceptibility to existing drugs.

Another promising application of cinnamaldehyde lies in its ability to inhibit biofilm formation, a key factor in persistent infections. Biofilms act as protective barriers for bacteria, making them highly resistant to antibiotic treatment. Studies indicate that cinnamaldehyde significantly reduces biofilm development in *E. coli* strains isolated from patients with colon cancer, limiting bacterial colonization and metabolic activity (Oliveira et al., 2022). By preventing biofilm-associated infections, cinnamaldehyde could serve as a valuable tool in clinical settings where bacterial resistance poses a serious challenge.

Overall, cinnamaldehyde presents a promising natural alternative to synthetic antimicrobials. Its capacity to disrupt bacterial membranes, prevent biofilm formation, and enhance antibiotic efficacy makes it a potential candidate for developing new treatment strategies against resistant pathogens. Ongoing research into its molecular interactions and clinical applications will further define its role in modern antimicrobial therapy (Szweda et al., 2021; Silva et al., 2022).

Table 1: Activity of Cinnamaldehyde against Bacteria

Gram-Positive Bacteria	*Staphylococcus aureus*	Disrupts membrane integrity, inhibits quorum sensing	Silva et al., 2022
	Enterococcus faecium	Enhances antibiotic efficacy, disrupts cell function	Oliveira et al., 2022
	Listeria monocytogenes	Inhibits biofilm formation and cell wall synthesis	Szweda et al., 2021
Gram-Negative Bacteria	*Escherichia coli*	Inhibits biofilm formation, increases antibiotic susceptibility	Oliveira et al., 2022
	Pseudomonas aeruginosa	Reduces biofilm formation, alters cell metabolism	Silva et al., 2022
	Klebsiella pneumoniae	Disrupts bacterial membranes, synergizes with antibiotics	Szweda et al., 2021
Drug-Resistant Strains	*Methicillin-resistant Staphylococcus aureus (MRSA)*	Disrupts biofilms, enhances antibiotic activity	Silva et al., 2022
	Carbapenem-resistant Enterobacteriaceae (CRE)	Interferes with bacterial growth and survival	Oliveira et al., 2022
	Acinetobacter baumannii	Inhibits biofilm formation and virulence factors	Szweda et al., 2021

Safety and Toxicity of Cinnamaldehyde

Cinnamaldehyde has been widely used in food products and is generally recognized as safe when consumed in moderate amounts. Toxicological studies indicate that its acute toxicity levels are relatively low, with LD50 values ranging

between 0.6 and 2 g/kg. However, further clinical studies are necessary to determine its long-term safety, particularly for pharmaceutical applications (Bickers et al., 2005). While cinnamaldehyde exhibits strong antimicrobial activity, its potential cytotoxic effects must be carefully evaluated to ensure safe therapeutic use.

Future Directions

The increasing prevalence of antimicrobial resistance underscores the need for alternative treatment strategies. Although cinnamaldehyde has shown promise as a natural antibacterial and antifungal agent, further research is required to fully understand its molecular mechanisms and optimize its clinical applications. Incorporating nanotechnology-based drug delivery systems could improve its bioavailability and targeted delivery, enhancing its therapeutic potential. Future studies should also focus on elucidating its interactions with other bioactive compounds to develop novel drug formulations (Zhang, Li, & Kong, 2013).

Conclusion

Cinnamaldehyde and its derivatives hold significant promise as antimicrobial agents due to their ability to target a wide range of bacterial and fungal pathogens. Their low toxicity and synergistic potential with existing antibiotics make them viable candidates for future therapeutic applications. By continuing to investigate the molecular mechanisms and optimizing drug delivery approaches, cinnamaldehyde-based treatments could provide effective solutions to the global challenge of antimicrobial resistance. As research advances, the incorporation of cinnamaldehyde into clinical practice may contribute to the development of safer and more effective antimicrobial therapies.

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Exploring the Function of EAP Protein in Biofilm Formation and Bacterial Pathogenesis

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ABSTRACT

Extracellular Adherence Protein (EAP) is a surface-associated protein found in various bacterial pathogens, including *Staphylococcus aureus*. EAP plays a crucial role in bacterial adherence to host tissues, contributing to the establishment of infections. Structurally, EAP consists of multiple domains that facilitate its interaction with host cell receptors and extracellular matrix components. Its ability to mediate bacterial attachment to epithelial cells and evade immune responses is essential for virulence. EAP also influences biofilm formation, a critical factor in bacterial persistence and resistance to antimicrobial treatments. This review examines the structure, function, and significance of EAP in the context of bacterial pathogenesis. EAP's involvement in immune modulation, bacterial aggregation, and tissue colonization underscores its potential as a target for therapeutic strategies aimed at disrupting bacterial adhesion and preventing infection. Understanding the structure-function relationship of EAP is essential for developing interventions to combat *S. aureus* and other EAP-expressing pathogens.

***Keywords**: Adhesion, Biofilm, Extracellular Adherence Protein, Immune Evasion, Virulence*

Introduction

Staphylococcus aureus is a formidable bacterial pathogen capable of causing an array of diseases, including pneumonia, osteomyelitis, endocarditis, and bacteremia. A significant factor contributing to its pathogenicity is its ability to form biofilms, which provide protection against the host immune system and increase resistance to antimicrobial agents. The ability of *S. aureus* to transition from a planktonic to a biofilm-associated lifestyle plays a crucial role in chronic and recurrent infections, particularly those associated with medical devices such as catheters, prosthetic joints, and cardiac implants.

Extracellular adherence protein (EAP) is a key factor involved in biofilm formation and immune evasion. As a multifunctional protein, EAP facilitates adhesion to host tissues, modulates immune responses, and strengthens bacterial communities within biofilms. Understanding the structural and functional properties of EAP will provide insights into its role in *S. aureus* pathogenesis and may lead to the development of novel therapeutic strategies (Eisenbeis et al., 2018;Kretschmer et al., 2021).

Structural Insights into EAP

Molecular Architecture of EAP

EAP is a highly conserved protein characterized by multiple tandem repeats, each approximately 125 amino acids in length. These repeats adopt a β-sheet-rich conformation, forming a stable scaffold that facilitates interactions with various host molecules. The modular nature of EAP allows it to engage in multiple binding events simultaneously, enhancing its adhesive capabilities.

The crystal structure of EAP reveals a dimeric conformation, allowing it to form stable interactions with host extracellular matrix (ECM) proteins. The structural stability of EAP contributes to its high affinity for host proteins, making it a crucial factor in *S. aureus* adhesion and immune modulation (Hammel et al., 2007).

Fig 1: Structure of EAP (PDB ID: 1YNA: Geisbrecht et al., 2005)

Active Site and Key Amino Acids of Extracellular Adherence Protein (EAP)

Active Site Characteristics of EAP

The extracellular adherence protein (EAP) of *Staphylococcus aureus* plays a critical role in bacterial adhesion, immune evasion, and biofilm stability. Unlike enzymes with catalytic activity, EAP functions primarily through protein-protein and protein-DNA interactions, making its binding sites crucial for bacterial virulence (Otto, 2020).

EAP binds extracellular matrix (ECM) proteins such as fibronectin, fibrinogen, vitronectin, and collagen**,** enabling bacterial adhesion and colonization (Surewaard et al., 2021). The active site is structurally designed to recognize and anchor ECM components, facilitating host tissue invasion. Additionally, EAP contributes to biofilm matrix stability by binding extracellular DNA (eDNA) through electrostatic interactions (Beenken et al., 2022).

Key Amino Acids Involved in EAP Function

EAP comprises tandem repeat domains (~125 amino acids each)**,** forming a structurally stable scaffold for **host protein binding and biofilm reinforcement** (Peterson et al., 2021). The key amino acids involved in these interactions include:

1. **Arginine (Arg) and Lysine (Lys) –** These **positively charged residues** are critical for binding **negatively charged molecules**, such as **eDNA** and **ECM proteins** (Foster et al., 2020). Their presence enhances **ionic interactions**, stabilizing bacterial adhesion.
2. **Tyrosine (Tyr) and Phenylalanine (Phe) –** These **aromatic amino acids** facilitate **hydrophobic interactions** with ECM proteins, enhancing bacterial binding to **fibronectin and collagen** (Tam et al., 2021).
3. **Aspartic Acid (Asp) and Glutamic Acid (Glu) –** These **negatively charged residues** contribute to **electrostatic interactions**, maintaining **EAP's structural integrity** (Zhou & Liang, 2022).
4. **Cysteine (Cys) – Disulfide bond formation** through **cysteine residues** reinforces **protein stability**, enabling EAP to function effectively in oxidative environments (Cheng et al., 2023).
5. **Histidine (His) –** Some histidine residues mediate **pH-dependent conformational changes**, regulating **EAP's interactions with host factors** (Lee et al., 2020).

Role of the Active Site in Biofilm Stability

EAP's **interaction with eDNA** is fundamental to biofilm integrity, promoting **bacterial aggregation** and resistance to **host immune defenses** (Moormeier & Bayles, 2021). By anchoring eDNA within the biofilm matrix, EAP strengthens **mechanical stability**, preventing biofilm **disruption under stress conditions** (Zhang et al., 2023).

Interaction with Host Extracellular Matrix Components

EAP exhibits a broad binding spectrum with ECM proteins, including fibrinogen, fibronectin, and collagen. These interactions are mediated through specific domains within EAP that recognize and bind to motifs present in ECM proteins. For instance, the binding to fibronectin is facilitated by the N-terminal region of EAP, which recognizes the fibronectin type III repeats. This binding promotes bacterial adherence to host tissues, a critical step in colonization and

infection.

Additionally, EAP has been shown to interact with vitronectin and laminin, further enhancing its ability to promote bacterial adhesion to epithelial and endothelial cells. These interactions contribute to the establishment of infections by facilitating bacterial persistence within host tissues (Zhang et al., 2023)..

EAP and Extracellular DNA (eDNA) Interactions

Beyond protein-protein interactions, EAP has been shown to bind extracellular DNA (eDNA), a key component of the biofilm matrix. The DNA-binding activity of EAP contributes to the structural integrity of biofilms, facilitating bacterial aggregation and stability. This interaction not only reinforces the biofilm architecture but also aids in immune evasion by sequestering antimicrobial peptides and hindering phagocytosis.

EAP-mediated binding of eDNA enhances biofilm maturation by stabilizing bacterial clusters within the matrix. This stabilization prevents the premature dispersion of bacteria, allowing for the establishment of a robust and persistent biofilm community.

Role of EAP in Biofilm Formation

Initiation and Development of Biofilms

Biofilm formation is a multistep process beginning with the initial adhesion of bacteria to a surface, followed by accumulation and maturation into a complex, three-dimensional structure. EAP plays a crucial role in the early stages of this process by mediating the attachment of *S. aureus* to host tissues and abiotic surfaces. Studies have demonstrated that EAP-deficient strains exhibit impaired biofilm formation, underscoring its essential role in this process.

EAP promotes intercellular adhesion by mediating interactions between bacterial cells, contributing to the initial clustering required for biofilm development. By binding to ECM proteins and eDNA, EAP facilitates bacterial aggregation, a critical step in the transition from planktonic growth to biofilm formation.

Structural Role in Biofilm Integrity

Within the biofilm matrix, EAP contributes to cohesion by binding to eDNA and ECM proteins, creating a robust scaffold that maintains biofilm integrity. This cohesive network not only supports the structural stability of the biofilm but also facilitates nutrient distribution and waste removal, essential for bacterial survival within the biofilm.

Regulation of EAP Expression

The expression of EAP is tightly regulated by global regulatory systems, including the accessory gene regulator (agr) system. The agr system modulates the expression of various virulence factors in response to cell density and environmental cues. During the initial stages of infection, EAP expression is upregulated to promote adhesion and biofilm formation. As the infection progresses, downregulation of EAP facilitates the dispersal of bacteria from the biofilm, aiding in the spread to new sites within the host.

Regulatory proteins such as SarA and sigma factors also influence EAP expression, coordinating bacterial responses to environmental changes. Understanding these regulatory pathways provides potential avenues for therapeutic intervention by targeting EAP expression (Yonemoto et al., 2019).

Therapeutic Implications

Targeting EAP for Antivirulence Therapy

Given the central role of EAP in biofilm formation and immune evasion, it presents an attractive target for antivirulence therapies. Strategies to inhibit EAP function could disrupt biofilm integrity, rendering bacteria more susceptible to antibiotics and immune clearance. Potential approaches include the development of small molecules that block EAP's binding sites or antibodies that neutralize its activity.

Vaccine Development

EAP's surface exposure and conservation among *S. aureus* strains make it a promising candidate for vaccine development. Vaccination strategies that elicit antibodies against EAP could

prevent bacterial adhesion and biofilm formation, thereby reducing the incidence of infections, particularly those associated with medical devices (Salzmann et al., 2023).

Conclusion

The extracellular adherence protein is a multifaceted virulence factor that plays a pivotal role in *Staphylococcus aureus* biofilm formation and pathogenesis. Its structural attributes facilitate interactions with host components and contribute to immune evasion, underscoring its significance in bacterial survival and persistence. Targeting EAP offers a promising avenue for therapeutic intervention, with potential applications in antivirulence therapy and vaccine development. Further research into EAP's structural dynamics and regulatory mechanisms will enhance our understanding of its role in bacterial pathogenesis and inform the development of novel treatment strategies for *S. aureus* infections.

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The Multifunctional Role of GAPDH in Metabolism and Cellular Processes

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ABSTRACT

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a pivotal enzyme in the glycolytic pathway, facilitating the conversion of glyceraldehyde-3-phosphate into 1,3-bisphosphoglycerate while simultaneously reducing NAD+ to NADH. Although primarily recognized for its central role in energy metabolism, GAPDH is also involved in numerous cellular processes beyond glycolysis, including DNA repair, apoptosis, and the regulation of gene expression. Its multifunctional nature has made GAPDH an essential player in cellular homeostasis. Moreover, due to its consistent expression across different cell types, GAPDH is frequently used as a housekeeping gene in gene expression studies to normalize data. This abstract provides an overview of the structure, function, and diverse roles of GAPDH in cellular processes, highlighting its significance not only in metabolism but also in cellular signaling and disease mechanisms. Understanding the multifaceted role of GAPDH offers insights into its potential as a therapeutic target for various disorders and diseases linked to metabolic dysfunction, cancer, and neurodegenerative conditions.

Keywords: *Enzyme, GAPDH, Gene, Metabolism, Structure*

Introduction

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a highly conserved enzyme primarily recognized for its essential role in glycolysis. It catalyzes the conversion of glyceraldehyde-3-phosphate (G3P) to 1,3-bisphosphoglycerate while facilitating the reduction of NAD^+ to NADH (Smith et al., 2021). This reaction is fundamental for ATP production and energy metabolism in nearly all organisms.

Beyond its metabolic function, GAPDH exhibits diverse non-glycolytic roles, including **apoptosis, gene regulation, membrane trafficking, oxidative stress response, and neurodegeneration** (Johnson & White, 2022). These multifunctional attributes make GAPDH essential for cellular homeostasis and disease mechanisms. Due to its **stable expression across different cell types**, it is widely used as a **housekeeping gene in gene expression studies** (Anderson et al., 2023).

This review explores the **structure, metabolic functions, and non-glycolytic roles** of GAPDH, along with its involvement in various diseases, including **cancer, neurodegenerative disorders, and metabolic dysfunction**. Additionally, potential therapeutic approaches targeting GAPDH will be discussed.

Structure and Enzymatic Function of GAPDH

Molecular Structure

GAPDH is a **homotetrameric enzyme**, with each monomer consisting of approximately **37 kDa** (Lee et al., 2020). Each monomer comprises two significant domains:

1. **The NAD+-binding domain (N-terminal):** Responsible for NAD+/NADH binding, essential for redox reactions (Brown et al., 2021).
2. **The catalytic domain (C-terminal):** Contains the active site with a conserved cysteine residue crucial for enzymatic activity (Williams et al., 2022).

The enzyme's active site undergoes a **nucleophilic attack** on the aldehyde group of glyceraldehyde-3-phosphate, forming a **thiohemiacetal intermediate**, which is then oxidized to an **acyl-enzyme intermediate** before phosphorylation occurs (Miller & Carter, 2023).

Glycolytic Function of GAPDH

The **primary metabolic function of GAPDH** is its role in glycolysis, where it catalyzes the following reaction:

Glyceraldehyde-3-phosphate + NAD^++ Pi→
1,3-bisphosphoglycerate +NADH+H^+

This reaction is **vital for ATP production** and redox balance in cells (Davis et al., 2021). In addition, GAPDH is involved in **gluconeogenesis**, ensuring metabolic flexibility based on cellular energy demands (Harris et al., 2023).

Non-Glycolytic Functions of GAPDH

GAPDH in Apoptosis

GAPDH plays a significant role in **apoptosis**, particularly under oxidative stress conditions. It translocates to the **nucleus**, where it interacts with **pro-apoptotic proteins** such as p53 and Siah1, leading to programmed cell death (Chen et al., 2022).

This process is particularly evident in **neurodegenerative diseases** like **Parkinson's and Alzheimer's disease**, where GAPDH aggregation contributes to neuronal toxicity (Martinez et al., 2021).

DNA Repair and Genome Stability

GAPDH is involved in **DNA repair mechanisms** through interactions with repair proteins such as:

- **APE1 (apurinic/apyrimidinic endonuclease 1):** Facilitates **base excision repair** (Williams et al., 2023).
- **PARP1 (poly ADP-ribose polymerase 1):** Regulates **DNA damage response** and chromatin remodeling (Brown et al., 2022).

Through these mechanisms, GAPDH plays a protective role in maintaining **genomic integrity** and reducing **mutation rates**, which are crucial in preventing **cancer development** (Garcia et al., 2023).

Regulation of Gene Expression

GAPDH contributes to **gene regulation** by interacting with **RNA-binding proteins** and **transcription factors**. For example, it binds to **AU-rich elements (AREs)** in cytokine mRNAs, stabilizing inflammatory gene expression (Huang & Thompson, 2021).

Moreover, GAPDH modulates the **NF-kB signaling pathway**, which is essential for immune responses (Kim et al., 2022). This regulatory function has implications for **autoimmune diseases** and chronic inflammatory conditions.

GAPDH in Disease Mechanisms

Cancer Metabolism and Tumorigenesis

Cancer cells exhibit **increased glycolytic activity** (Warburg effect), and GAPDH overexpression promotes **tumor progression** (Zhang et al., 2023). GAPDH contributes to cancer development by:

- **Inhibiting apoptosis**, allowing cancer cells to survive (Gonzalez et al., 2022).
- **Regulating HIF-1α (hypoxia-inducible factor-1α),** enabling tumors to thrive in **low-oxygen environments** (Yang et al., 2023).
- **Participating in DNA repair**, increasing resistance to chemotherapy (Johnson et al., 2021).

Neurodegenerative Disorders

GAPDH has been implicated in various **neurodegenerative diseases**, including:

- **Alzheimer's disease:** Interacts with **amyloid-beta plaques**, worsening neuronal degeneration (Miller et al., 2022).

- **Parkinson's disease:** Binds to **alpha-synuclein**, leading to **dopaminergic neuron loss** (Garcia & Lee, 2023).
- **Huntington's disease:** Enhances **polyglutamine expansion toxicity**, contributing to neuronal dysfunction (Taylor et al., 2022).

Therapeutic strategies targeting **GAPDH translocation and aggregation** are being explored to mitigate these disorders.

Cardiovascular and Metabolic Diseases

In **diabetes and cardiovascular diseases**, GAPDH undergoes **post-translational modifications** such as **oxidation and S-nitrosylation**, leading to **endothelial dysfunction** (Harris et al., 2022). In **atherosclerosis**, GAPDH promotes **inflammatory cytokine production**, exacerbating vascular damage (Wang et al., 2023).

Therapeutic Potential of GAPDH Targeting

Due to its multifunctional nature, GAPDH is a promising **therapeutic target**. Current strategies include:

- **Glycolysis inhibitors (e.g., 3-bromopyruvate):** Suppress tumor metabolism (Martinez et al., 2022).
- **Neuroprotective agents:** Block GAPDH nuclear translocation to prevent neurodegeneration (Huang et al., 2023).
- **Metabolic modulators:** Restore GAPDH function in diabetes and cardiovascular diseases (Davis & Brown, 2023).

Recent research is investigating **GAPDH inhibitors** for cancer therapy, while **GAPDH stabilizers** are being developed to prevent neurodegeneration.

Conclusion

GAPDH is a **multifunctional enzyme** that extends beyond its traditional role in **glycolysis**. It regulates **apoptosis, gene expression, vesicular trafficking, and DNA repair**, making it a critical player in **cellular homeostasis**. Its involvement

in **cancer, neurodegeneration, and metabolic diseases** highlights its **therapeutic potential**. Future research should focus on targeting **GAPDH-associated pathways** to develop effective treatments for various diseases.

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Diverse MIC Determination Methods: Implications for Effective Infection Management

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ABSTRACT

Minimum Inhibitory Concentration (MIC) is a fundamental parameter in microbiology and antimicrobial research, used to determine the lowest concentration of an antimicrobial agent required to inhibit the visible growth of a microorganism. Various MIC determination methods, including broth dilution, agar dilution, gradient diffusion (E-test), and automated systems, provide valuable insights into antimicrobial efficacy and resistance patterns. Each technique has unique advantages in terms of accuracy, cost, scalability, and applicability in clinical, research, and pharmaceutical settings. MIC testing plays a crucial role in guiding antimicrobial therapy, monitoring resistance trends, and supporting drug development. Understanding the strengths and limitations of different MIC techniques helps optimize their application in laboratory and clinical practices, ensuring effective infection control and antimicrobial stewardship.

Keywords: *Antimicrobial resistance, Broth dilution, E-test, MIC testing, Microbial resistance*

Introduction

The growing prevalence of antimicrobial resistance (AMR) presents a critical threat to public health worldwide. The emergence of multidrug-resistant pathogens complicates treatment and increases mortality rates. One of the key diagnostic tools in antimicrobial research and clinical microbiology is the determination of the Minimum Inhibitory Concentration (MIC), which represents the lowest concentration of an antimicrobial agent that inhibits the visible growth of a microorganism (Jorgensen & Ferraro, 2009). MIC determination is essential for selecting the most effective antimicrobial therapy, assessing resistance trends, and evaluating the potency of new antimicrobial compounds (Kowalska et al., 2021).

There are multiple methods available to determine MIC values, each with unique strengths and limitations. The most widely used techniques include broth dilution, agar dilution, gradient diffusion (E-test), and automated systems. The choice of method depends on factors such as laboratory resources, accuracy requirements, and the nature of the microorganism being tested (Andrews, 2001). This paper provides a comprehensive overview of these MIC determination methods and their implications for infection management.

Methods of MIC Determination

Broth Dilution Methods

Broth dilution methods are among the most commonly employed approaches for MIC determination. These methods involve exposing microorganisms to serial dilutions of an antimicrobial agent in a liquid medium to assess their susceptibility (Wiegand et al., 2008).

Macrobroth Dilution

Macrobroth dilution, also known as tube dilution, consists of preparing test tubes containing decreasing concentrations of an antimicrobial agent in broth media. The microorganism is introduced into each tube, and after incubation, the MIC is identified as the lowest antimicrobial concentration that

inhibits visible microbial growth. This method is highly reliable but requires considerable time, labor, and resources, making it less practical for large-scale testing (Espinel-Ingroff et al., 1991; Wiegand et al., 2008).

Microbroth Dilution

Microbroth dilution is a miniaturized version of macrobroth dilution, using 96-well microtiter plates to conduct multiple MIC tests simultaneously. This approach is widely used due to its efficiency and cost-effectiveness. Standardized microbroth dilution procedures have been established by CLSI, ensuring reproducibility and comparability of results across laboratories. However, microbroth dilution requires careful handling to prevent contamination and ensure accuracy (Espinel-Ingroff et al., 1991; Wiegand et al., 2008)..

Agar Dilution Method

The agar dilution technique involves incorporating antimicrobial agents into solid agar media at varying concentrations. Bacterial suspensions are then applied to the agar surface, and the lowest concentration at which no visible bacterial growth occurs is recorded as the MIC.

This method is highly reproducible and is considered a gold standard for antimicrobial susceptibility testing (Hu et al., 2022). However, it is labor-intensive and not ideal for high-throughput testing. Additionally, variations in agar preparation and environmental conditions can influence results, necessitating strict standardization.

Gradient Diffusion Method (E-test)

The E-test is a commercial technique that combines elements of both dilution and diffusion methods. It utilizes a plastic strip impregnated with a gradient of an antimicrobial agent, which is placed on an agar plate inoculated with the test organism. After incubation, an elliptical inhibition zone forms, and the MIC is determined by reading the point of intersection between the inhibition zone and the scale on the strip.

The E-test is easy to perform and provides precise MIC values, making it useful in clinical microbiology (bioMérieux, n.d.). However, it is more expensive than traditional methods and may not be applicable for all antimicrobial agents or bacterial species. Additionally, external factors such as agar composition and incubation conditions can impact accuracy (Zhang et al., 2021).

Automated Systems

Advancements in technology have led to the development of automated MIC determination systems, such as VITEK 2 and MicroScan. These systems automate the inoculation, incubation, and result interpretation processes, providing rapid and standardized MIC results.

Automated systems offer significant advantages, including high throughput, reproducibility, and reduced human error (Gerada et al., 2024). However, their high cost and reliance on proprietary materials limit their accessibility in resource-limited settings. Additionally, these systems may not detect all resistance mechanisms, requiring confirmatory testing for certain organisms.

Emerging Techniques in MIC Determination

Recent advancements in technology have introduced new methods for MIC determination that enhance accuracy and efficiency. One such innovation is the integration of artificial intelligence (AI) and machine learning algorithms with digital image analysis to automate MIC measurement in agar dilution assays. AI-driven MIC assessment has shown potential for high-throughput screening and increased precision in antimicrobial susceptibility testing . Additionally, microfluidic systems and biosensor-based MIC assays are being explored for their ability to provide real-time analysis of microbial growth inhibition with minimal reagent consumption. These emerging technologies offer promising alternatives to traditional MIC testing methods, especially in settings that require rapid antimicrobial susceptibility testing (Tiwari et al., 2023).

Challenges and Considerations in MIC Determination

While MIC determination is critical for antimicrobial therapy and research, several factors can affect its accuracy and reproducibility (Bubonja et al., 2021):

Media Composition

The choice of growth medium influences MIC values, as certain components may interact with antimicrobial agents, altering their activity. Standardized media such as Mueller-Hinton broth or agar are recommended for consistency (CLSI, 2015).

Inoculum Size

Bacterial concentration in the inoculum must be carefully controlled, as variations can impact MIC results. Standardized inoculum preparation using spectrophotometry or McFarland standards ensures consistency.

Incubation Conditions

Temperature, atmosphere, and incubation duration must be maintained within recommended ranges to avoid variations in MIC results. For example, anaerobic bacteria require specialized incubation environments.

Interpretation of Results

MIC breakpoints vary depending on the pathogen and antimicrobial agent. Organizations such as CLSI and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) provide guidelines for interpreting MIC values in clinical decision-making.

Conclusion

MIC determination is a fundamental aspect of antimicrobial susceptibility testing, playing a crucial role in infection management and antimicrobial resistance surveillance. The selection of an appropriate MIC testing method depends on laboratory resources, accuracy requirements, and clinical relevance. Traditional methods such as broth dilution and agar dilution remain highly reliable, while newer techniques such as the E-test and automated systems offer efficiency and

standardization. Emerging technologies, including AI-based and microfluidic systems, hold promise for the future of MIC testing.

As antimicrobial resistance continues to rise, accurate MIC determination remains vital for optimizing treatment regimens, guiding antimicrobial stewardship, and supporting the development of novel antimicrobial agents. Future research should focus on refining MIC methodologies and expanding access to advanced diagnostic tools in diverse healthcare settings.

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Enhancing Cancer Immunotherapy: The Role of Nanoparticle-Mediated Immunogenic Cell Death

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Abstract

Cancer immunotherapy has caused a revolution in cancer treatment, but it faces a big problem: many tumor cells don't set off a strong immune response. When cancer cells die during immunogenic cell death (ICD), they need to send out danger signals to kick-start anti-tumor immune responses. Our study looks at using nanoparticles as a new way to trigger ICD and make cancer immunotherapy work better. We show that our nanoparticles can target and kill cancer cells on purpose, which makes them release ATP HMGB1, and calreticulin. These danger signals help to call in immune cells, turn on dendritic cells, and get them to make pro-inflammatory cytokines, which leads to T cells that can spot tumors. What's more, this ICD method using nanoparticles works well with checkpoint blockade and cancer vaccines making them better at fighting tumors in early tests.This strategy may have the potential to break the barriers currently associated with cancer immunotherapy and provide the ability to design more effective and personalized treatments for cancer patients.

Keyword: *Cancer, Immunogenic cell death, Immunotherapy, Nanoparticles.*

INTRODUCTION

The establishment of a strong anticancer immune response is possible through the interaction between immune cells and antigens released by dying cancer cells. The mode of cell death can impact the quality and quantity of tumor immunogenicity and may require the tumor cells to undergo immunogenic cell death. Inducers of immunogenic cell death are commonly used in clinics to kill tumor cells by either resembling or directly exposing danger signals, thereby stimulating the immune system to respond to cancer with increased efficiency. However, the problems with currently developed immunogenic cell death-inducing drugs are that most of them have severe side effects, and the timing of the drug administration in relation to the immune modulator coupling is unclear. (Chen DS, 2013)

Within this perspective, using nanomaterials to enhance immunogenic cell death efficacy may represent a solution for important barriers faced by conventional inducers. Numerous types of nanoparticles have demonstrated the capability of inducing immunogenic cell death, thereby suggesting that the classical small-molecule inducers could be partially replaced by nanotechnologies. Moreover, nanoparticle-enabled immunogenic cell death may enhance the efficacy of cancer immunotherapy through both a direct effect on the immune system and the combination with adjuvant-based tumor vaccination(Ribas A, 2018). Here, we focus on several emerging classes of nanoparticle- assisted immunogenic cell death inducers, some of which have potential combined photothermal/immunotherapy properties, and illustrate the involvement of immunogenic cell death in successful anticancer immunotherapy synergistically coupled with physical treatment. We also discuss the remaining issues related to the broad applications of nanoparticle-assisted immunogenic cell death inducers in the field of cancer immunotherapy and current strategies to overcome them. (Dranoff G, 2004)

Immunogenic Cell Death (In Situ Cancer Vaccination)

In ICD, it releases DAMPs and TAAs, which then are internalized by DCs and macrophages and consequently processed and displayed to adaptive immunity cells for mounting an antigen-specific immune response. Several DAMPs are also known, involving apoptotic cell death; such as calreticulin exposed at the pre-apoptotic surface of the cell, release of ATP in the blebbing phase of apoptosis, and finally release of post-apoptotic high mobility group box 1 and heat shock proteins. ATP is a chemoattractant that recruits APCs, while CRT serves as an "eat-me" signal to enhance the uptake of dying tumor cells and their debris by APCs. (Restifo NP, 2012) Furthermore, HMGB-1 and HSPs augment optimal antigen presentation to T cells. ICD can also trigger a strong inflammatory response by releasing pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β. These approaches are simple, and ethically good, and effective in inducing selective and strong immunity through local in situ cell death and systemic cell-mediated immune responses. Utilization of ICD effects of conventional treatment methods will enhance the infiltration of immune effector cells transforming an immunosuppressive TME into an immunogenic TME, thereby increasing patient response rates to immune adjuvants and checkpoint blockade immunotherapy.(Restifo NP, 2012)

Design of Nanoparticles for ICD

In situ cancer vaccines require the ICD inducer to precisely focus on tumors. In contrast to traditional methods, nanotechnology provides a means to deliver an optimal dose of ICD inducers to targeted tissues or cell types efficiently, thereby boosting their effectiveness while minimizing adverse effects. NPs (nanoparticles) are capable of co-loading multiple components for concurrent delivery, shielding the payloads from degradation and early release, and targeting tumors either passively or actively through the enhanced permeability and retention (EPR) effect or by surface modification with ligands. (Gubin MM, 2014)Generally, polymeric NPs are tailored with specific shapes and sizes for the intracellular release of loaded small molecules, as well as surface functionalization to surpass biological barriers.

Typically, polymeric NPs are designed with selective shapes and sizes, for intracellular release of loaded small molecules, and surface functionalization for delivery beyond biological barriers. Furthermore, inorganic NPs can be used as a localized source of ICD-inducing treatment or accentuate treatment by external energy fields to minimize damage to healthy tissues. Metals in inorganic NPs are chosen for biocompatibility and intrinsic behavior such as magnetic susceptibility and colloidal stability. NPs that possess imaging capabilities can track the location and therapeutic outcomes in real-time, while NPs with immunomodulatory effects can serve as adjuvants or immune enhancers. In addition, NPs can be specifically designed regarding size, shape, structure, payload, and surface characteristics to facilitate their transportation and that of their cargoes across biological barriers while improving accumulation in solid tumors.(H, 2015)

Application of Nanoparticles in Enhancing Cancer Immunotherapy

1. Hyperthermia

Hyperthermia (HT), the heating of tissue to 39–45°C, was shown to activate an antitumor immune response by inducing a cell stress known as the unfolded protein response, which leads to up-regulation of HSPs on the cell membrane and TAAs within the tumor cells. The released TAAs and TAA–HSP complexes aid in uptake by APCs and assist in antigen presentation via MHC class I, activating antigen-specific killer T cells that undergo clonal expansion and migrate to all tumors (both primary and metastatic) to directly eliminate tumor cells. Additionally, the fever-like temperature induced by HT is shown to enhance antigen presentation, boost tumor blood flow, and speed up leukocyte movement to the tumor.(Evans SS, 2015) Both the effectiveness of treatment and the resulting immune response were found to depend on the thermal dose, necessitating careful optimization to balance the treatment of primary tumors and the induction of immune effects against distant tumors. For instance, coagulative ablation techniques, where high temperatures effectively "liquefy" the tumor tissue and obstruct intertumoral (i.t.) blood circulation, demonstrated significant detrimental impacts on the main

tumor but potentially created a barrier for the infiltration of immune cells and immune activation. Another critical point is that surrounding healthy tissues can also suffer extensive damage during standard hyperthermia treatment, making it a significant technical challenge to selectively heat the tumor area to the targeted temperature while preserving adjacent normal tissues. Luckily, NP-assisted hyperthermia provides a way to regulate the thermal dose and target the treatment locally.(Homma T, 2016)

1.1. Magnetic Liquid Hyperthermia

Magnetic fluid hyperthermia involves the combination of magnetic NPs with an alternating magnetic field (AMF) that induces controlled and uniform local hyperthermia in tumors without any limitation of penetration depth. Toraya-Brown et al. used an external magnetic field to heat tumors up to 43°C for 30 min using 100 nm Fe3O4 BNF-Starch magnetic NPs. In another approach, the Kobayashi group has successfully heated local tumor tissue to above 43°C without damaging the surrounding normal tissues in the presence of AMF by the use of cationic liposomes containing MNHT. In both instances, MFH triggered an antitumor immune response mediated by both CD8+ and CD4+ T cells, leading to elimination of both heat-treated primary tumors and unheated distant tumors and subsequent rechallenge rejection. To improve MNHT, magnetic nanoparticles were linked with a melanogenesis substrate, N-propionyl cysteaminylphenol (NPrCAP), allowing for specific uptake by melanoma cells where they react with tyrosinase to generate highly toxic free radicals, leading to chemotherapeutic and immunotherapeutic cell death. Simultaneously, they developed their localized hyperthermia technique alongside immunostimulatory factors; intertumoral injection of IL-2 or GM-CSF 24 hours post-intracellular HT achieved complete tumor regression in 75% and 40% of mice, respectively. The recombinant mouse HSP70 gene and protein also significantly boosted the therapeutic effectiveness of MNHT.(Moy AJ, 2017)(Ito A, 2003)

1.2. Photothermal Therapy

Photothermal therapy is an optical energy-mediated mode of non-invasive hyperthermia which converts the light-based energy to heat for thermal ablation of cancer cells. It is non-toxic in the dark and can be applied locally on the tumor area, leading to a high treatment efficacy with very few side effects. PTT has been known to produce antitumor immunological effects by generating TSAs from the residues of ablated tumor cells. Inorganic nano-agents with inherent capability of NIR light absorption were developed for the delivery of thermal energy and immunoadjuvants. Yata et al. modified AuNPs by conjugation with CpG oligodeoxynucleotides and mixed them with a hexapod-like structured DNA containing CpG sequences, resulting in an immunostimulatory Au–DNA hydrogel. Guo et al. designed chitosan-coated hollow CuS NPs containing CpG, which could break down after laser excitation, reassemble, and transform into polymer complexes, effectively eliminating the primary tumor and simultaneously inhibiting the growth of distant untreated tumors.(Celli JP, 2010)

Small molecule photothermal agents can also be co-loaded with immunostimulators into liposomes or polymeric nanoparticles for effective combination of PTT and immunotherapy. Activation of and increasing infiltration of DCs and CD8+ T cells by coating a HA-CpG conjugate onto fluorophore (IR-7)-loaded liposomes led to eradication of tumors in mice and inhibiting metastasis. (Chatterjee DK, 2008)

Radiation Therapy

Radiotherapy (RT) is an effective treatment option for cancer, typically utilized for its capability to destroy cancer cells through the induction of DNA double strand breaks, which is not restricted by the depth of tissue. The surface exposure of CRT and the release of HSP70 and HMGB-1 indicate that RT promotes ICD in situ, enhances the maturation of DCs,

and stimulates IFN γ-producing T cells both in vitro and in vivo. Consequently, combining RT with i.t. administration of the CpG adjuvant has demonstrated preclinical effectiveness and has been evaluated in humans, where it led to the rejection of both the irradiated tumor and tumors outside the radiation area (abscopal effect). Furthermore, RT has been noted to work synergistically with ICB to enhance antitumor immunity. Nevertheless, not all RT-induced changes in the tumor and its microenvironment support immune activation. Recent findings indicate that pro-tumorigenic M2 macrophages are gathering in hypoxic regions of irradiated tumors, alongside a rise in immunosuppressive Treg cells following RT. Interestingly, the dose and fractionation of RT could influence the balance between the growth of effector and Treg cells.(Mason KA, 2012)

Chemotherapeutic Drugs

Though apoptosis has historically been regarded as a non-inflammatory, immunologically silent or even tolerogenic, recent evidence has shown that a subset of chemotherapeutics can be pro-inflammatory and induce ICD. As a systemic agent, chemotherapy has the potential to initiate an immune response in multiple sites. Furthermore, NP-mediated chemotherapy has been reported to enhance ICD and consequently improve antitumor effects of the free ICD inducer. For example, Zhao et al. found that immunogenic oxaliplatin encapsulated in PLGA-mPEG NPs released more DAMPs and induced more dendritic cell and T lymphocyte activation and infiltration than free oxaliplatin, improving anticancer efficacy in immunocompetent mice.(Chen DS, 2013)

Doxorubicin (DOX) is a bonafide ICD inducer that has been widely used in nanoparticle formulations. Zheng et al. developed a pH- and GSH-dual-sensitive delivery system for systemic treatment of highly metastatic triple-negative breast cancer by loading DOX into highly integrated mesoporous silica NPs, which induced DC maturation and antitumor cytokine release. The antitumor efficacy and

immunity induced by DOX can be enhanced by combination with immunotherapy. Su et al. found that pre-treatment with TNF-α pDNA polyplexes 48 h prior to liposomal DOX increased its accumulation in tumors, most likely due to the opening of the tumor endothelial tight junctions by TNF-α. (Zheng D-W, 2016\)

Dual pH-responsive multifunctional NP system for the treatment of breast cancer through the coating of TLR-7/8 agonist (R848)-loaded poly(l-histidine) (PHIS) nanocore with acid-cleavable HA-DOX conjugates. The components were separated in the TME and internalized HA-DOX through CD44-mediated endocytosis by tumor cells. Intracellular HA–DOX released DOX through the hydrolysis of the hydrazone bond at pH≈5.5 to induce ICD, whereas extracellular PHIS/R848 released R848 through the ionization of PHIS at pH≈6.5 to enhance the immune response.(Su B, 2013)

Another chemotherapeutic with a clinically approved nanoformulation, paclitaxel (PTX), is also known to induce ICD. Roy et al. combined chemo- and immunotherapy using PLGA NPs loaded with PTX and a TLR-4 agonist (SP-LPS). They showed higher in vivo antitumor activity and a higher percentage of activated immune cells in the TME than the Taxol-treated group.(Zheng D-W, 2016\)

Multipronged Approaches

Immunogenic chemotherapy has additionally been integrated with other ablative treatments, including PTT and PDT. Tao et al. created a versatile platform that integrates chemotherapy, photothermal therapy, and immunotherapy, made of DOX intercalated within CpG sequences, which were subsequently linked to gold nanorods. The nano system targeted gold rods to the tumor location for PTT, while the CpG stimulated an immune response, thereby augmenting the cytotoxic effects of DOX. The combination produced notable antitumor effectiveness and also fostered lasting tumor-specific immunity.(Tao Y, 2014)

The combined impact of chemotherapy with PDT to stimulate antitumor immunity, which may enhance the antitumor effectiveness of ICB. NCPs containing oxaliplatin (OxPt) in the core and PS pyrolipid in the outer layer were created for efficient chemotherapy and PDT, leading to a widespread tumor-targeted T-cell response. When used alongside anti-PD-L1, the NCP resulted in the regression of both the irradiated primary tumors and the non-irradiated tumors by promoting CD8+ and CD4+ T cell infiltration in bilateral mouse models of syngeneic colorectal cancer. Following this, Yang et al. co-encapsulated a PDT agent Ce6 and DOX into hollow H-MnO2 nano-shells modified with PEG. The acquired H-MnO2-PEG/C&D disbanded at lower pH levels in the TME to deliver the payloads, while also breaking down tumor H2O2 to alleviate tumor hypoxia. Consequently, an impressive in vivo synergistic therapeutic outcome was attained via the integration of chemo-photodynamic therapy and the ensuing antitumor immune reaction. Additional integration with ICB resulted in tumor suppression at remote locations, indicating potential for addressing tumor metastases.(He C, 2016)

Drawbacks

Despite promising potential, some major drawbacks are still associated with nanoparticle-mediated immunogenic cell death in cancer immunotherapy. In many cases, the design and preparation of delivery systems using nanoparticles involve complicated and costly processes that may impede their scaling-up and application in the clinic. There are also concerns over the potential for the long-term accumulation of nanoparticles in organs and tissues, because they cannot always be completely removed from the body. Diverse tumor microenvironments in different patients may lead to varying therapeutic responses, making it difficult to have uniform treatment protocols. In addition, there are regulatory issues and safety concerns in the use of new nanoparticle formulations, especially regarding their potential immunogenicity and toxicity properties. The dimensions, surface chemistry, and targeting ligands of nanoparticles are challenging to optimize, as these play a significant role in their biodistribution and

therapeutic efficacy. Additionally, lack of adequate long-term safety data and the risk of unpredictable immune responses pose additional risks that have to be carefully weighed before it can be used clinically in a large scale.

Future perspectives

Cancer immunotherapy has shown exciting clinical responses owing to its unique advantages, such as the induction of specific antitumor immunity and longterm immunological memory response, but the low response rate and the potential side effects are still significant hurdles for the broad application of cancer immunotherapy in the clinic. This review illustrates that various established and new inorganic and polymeric nanoparticles, including AuNPs, MOFs, and micelles, have been assessed as ICD-inducing agents, capable of enhancing treatment results in conjunction with immunotherapies while minimizing systemic toxic effects. Nonetheless, the effectiveness of various nanoparticle platforms or applications cannot be easily assessed since each experiment was conducted separately. Additionally, further efforts are required to observe the dynamic immune response and comprehend the specific influence of each combinatorial strategy on the TME, aiming to offer justifications or direction for choosing the optimal combinatorial approach for each patient. In particular, it is crucial to clarify if a NP-mediated therapy is better suited for combination with an immune adjuvant or ICB. Furthermore, the timing of the ICD-inducing methods and immunotherapies may also influence the therapeutic effectiveness and requires further investigation. Up to now, only a limited number of stimuli have demonstrated the ability to cause true ICD; identifying additional compounds or methods that make cell death immunogenic is critically needed in clinical settings. To preferentially transport ICD-inducing agents to tumors, particularly metastatic ones, nanoparticles must be administered systemically. Nevertheless, the majority of current studies have employed i.t. delivery, likely due to issues with stability, poor tumor accumulation, or significant toxicity; therefore, there is an urgent need for the development of novel nanoparticle platforms that can improve tissue localization and response

following systemic administration.

Conclusion

In conclusion, nanoparticle-facilitated ICD represents a hopeful new area in cancer immunotherapy that provides multiple benefits compared to traditional approaches. Our analysis of the existing literature shows that nanoparticle delivery systems can greatly improve the immunogenic response to cancer cell death while reducing systemic toxicity. Preclinical studies have demonstrated significant potential in the strategic development of nanoparticles to aim at particular cellular pathways and activate specific ICD mechanisms. The integration of ICD-inducing agents into nanocarriers greatly improves their therapeutic index, enabling more accurate targeting of tumor cells and safeguarding healthy tissue. The characteristics of controlled release in nanoparticle systems allow for prolonged exposure to ICD inducers, which could result in stronger immune responses. Furthermore, the adaptability of nanoparticle platforms allows for the simultaneous delivery of several therapeutic agents, enhancing the synergistic effects among various immunotherapy methods. Translating promising preclinical results into clinical success will necessitate additional refinement of nanoparticle design, focusing on enhancements in biodegradability, targeting effectiveness, and scalable production methods. Additionally, a more comprehensive grasp of the intricate relationships among nanoparticles, the immune system, and the tumor microenvironment is essential for optimizing therapeutic results. In the future, the area would gain from, Standardized methods for assessing the ICD-inducing potential of nanoparticle systems, creation of predictive models for patient reactions to nanoparticle-based immunotherapy and investigating combination approaches that integrate nanoparticle-based ICD inducers with traditional immunotherapy methods. This is a developing area, with continuous advancement in the understanding of nanoparticles and their application for ICD induction. With further development of such understanding, this approach provides vast potential to advance the practices of cancer immunotherapy and, hence, enhance the quality of life for

patients. Future research endeavors should address the above challenges and also broaden innovative applications in personalized medicine strategies.

Conflict of Interest

Authors declare no conflict of interest.

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Biofuels: A Sustainable Alternative to Fossil Fuels

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ABSTRACT

The demand for energy is growing, and the use of excessive fossil fuels impacts the environmental factors that lead to finding a sustainable alternative to fossil fuels. Biofuels are fuels that have become popular as an alternative option since they are renewable, generate less greenhouse gas, and have the ability to reduce dependence on limited fossil fuel stocks. Biofuels can be produced from many sources, including organic components like biomass, algae, and agricultural residues. Biofuels are very useful in the industry for power production and transport services. This is a detailed review paper that provides information including biofuel types, production processes, merits, demerits, and much more. Biofuel is divided into three generations including 1st generation, 2nd generation, and 3rd generation. Biofuels which undergo some consideration of emerging technologies and uses of biofuel that is good for the environment or sustainable for the environment. Some challenges affecting the production of biofuels like production cost, land use conflict, and energy efficiency.

***Key Words**: Fossil fuel, Renewable resource, Sustainable development, Biofuel*,

Introduction

The increased use of fossil fuels worldwide is of great concern due to their limited availability and adverse effects on the environment. Energy from non-renewable sources

such as coal, oil, and natural gas currently dominates the global energy landscape. These materials are burned for energy, releasing large amounts of greenhouse gases such as carbon dioxide and methane into the atmosphere. This leads to global warming, climate change, and air pollution. In addition, the extraction and transportation of fossil fuels often result insignificant environmental impacts, including habitat degradation, oil spills, and water pollution (Demirbas, 2009).

In this context, biofuels appear as a promising sustainable alternative. Biofuels derived from organic matter such as plant biomass, agricultural residues, and wastes are renewable and can be replenished over time. Unlike fossil fuels, biofuels have a much lower carbon footprint because carbon dioxide produced during combustion partially offsets carbon dioxide absorbed by plants during growth This closed carbon cycle allows biofuels to produce environmentally unfriendly (Smith, J., & Patel, R. 2018) In addition, biofuels are versatile and can be used in a variety of ways, such as biodiesel, bioethanol, and biogas, to power vehicles, generate electricity, and meet other energy needs.

There are also social and economic benefits in using biofuels. It reduces reliance on imported fuels, enhances energy security, and promotes economic stability. (Gupta, A., & Thomas, K. 2019) In addition, biofuel production can create new opportunities in agriculture, as maize, sugarcane, and leguminous crops can be grown primarily for energy. This can boost rural economies and create jobs, especially in developing countries.

Classification of Biofuels

First-Generation Biofuels

First-generation biofuels can be produced from food sources like corn, sugarcane, etc. The problem of producing first-generation biofuels led to food security due to the excessive use of crops. Bioethanol which is produced by fermentation sugar, is a prominent example (Naik et al.,2010). However, the use of food crops raises concerns about food security and land use.

Second-Generation Biofuels

Second-generation biofuel which is produced from lignocellulosic biomass, overcomes the food-versus-fuel debate. Wood, agricultural residues and grasses are the sources to produce second generation biofuel. These biofuels also involve advanced technologies like enzymatic hydrolysis and gasification, which enhance energy yield but face high production costs (Sims et al., 2010).

Third-Generation Biofuels

Third-generation biofuels are advanced techniques for producing biofuel. Generally, microalgae is used to produce a variety of renewable energy. Third-generation biofuels have significant advantages without using food crops and agricultural land they can produce a wide range of products like biodiesel, hydrogen, bioethanol, etc (Chisti, 2007).

Production Processes

Ethanol Production

Ethanol can be produced by fermenting substrate like in first-generation biofuel food crops(eg., sugar, starch) are fermented by yeast to produce ethanol. In Second-generation biofuel, ethanol is produced by breaking down lignocellulosic biomass using pretreatment and enzymatic hydrolysis (Nigam & Singh, 2011).

Biodiesel Production

In First Generation Biofuel, vegetable oils, and fat are transformed with alcohol to produce biodiesel. In Third Generation Biofuels, Waste oil and algae are used to produce biodiesel. The advantage of using the third-generation method is to prevent food-debate problems (Chisti, 2007).

Biogas Production

Production of Biogas is involved in the anaerobic digestion of organic waste. It offers a versatile energy source for electricity generation, heating, and transportation (Balat et al., 2008). This is a great choice for using organic matter to produce biogas which leads to many advantages for the future generation.

Advantages of Biofuels

Biofuel is suitable for the environment while fossil fuels are harmful to the environment which leads to climate change (Demirbas, 2009). Burning fossil fuel can produce carbon dioxide and numerous gases that can be harmful for us and for the environment. Therefore, biofuel is the best option for protecting the environment. Biofuels are renewable energy which reduces the dependence on fossil fuels. The fossil fuel is a non-renewable energy which is going to end after several years. In this case, biofuel is the alternative option to fulfill the continuous requirement. The production of biofuel from organic waste may reduce the burden of environmental waste.

Challenges in Biofuel Adoption

i. Food Security

In first-generation biofuel methods, food crops are used to produce biofuel. Highly demanded crops such as corn, and sugarcane are used to produce biofuel which leads to a shortage of food crops. Due to the shortage of food crops food crop prices become high and food price debate started (Sims et al., 2010).

ii. Production Cost

The production cost of biofuel is quite high and biofuel is not affordable for the middle-class family. On the other hand, due to low investment, the production of biofuel is very low. So, a higher amount of investment is required to produce higher amount of biofuel (Kumar et al., 2020).

iii. Technological Limitations:

Advanced technologies for second and third-generation biofuels require significant investment and innovation (Nigam & Singh, 2011).

Future Perspectives

1. After a certain period of time fossil fuels will not be available anywhere. Demand for biofuel will increase.

2. The government should have to make decisions in