Review Article

Delving into the Fascinating Realm of Biofilm Forming Bacteria: A Review

Abstract

Bacterial biofilms are complex, three-dimensional communities of microorganisms that are encased in a self-produced extracellular matrix, which allows them to adhere to various surfaces, including medical devices, industrial equipment and living tissues. These biofilms exhibit remarkable structural and functional heterogeneity, genetic diversity and complex community interactions. Biofilm formation is a dynamic process that is triggered by environmental changes and involves multiple regulatory networks that mediate gene expression changes. Biofilms can confer significant advantages to the bacteria, such as increased tolerance to antimicrobial agents, evasion of the host immune system, and facilitation of horizontal gene transfer. Chronic nosocomial infections in clinical settings are often caused by biofilm. This review provides a concise overview of the development, dispersal and therapeutic strategies for bacterial biofilms, with a focus on their medical significance and the challenges they pose in the dawn of the post-antibiotic era. It focuses on emerging therapeutic strategies such as anti biofilm agents, phage therapy, use of nanoparticles, anti virulence strategies such as quorum sensing inhibitors, use of combination therapies, photo dynamic therapy, gene editing and RNA based therapies, immunotherapy, natural product based therapies, surface modification of meducal devices etc.

Keywords: Antimicrobial resistance, Chronic infections, Extracellular polymeric substances, Immune Evasion, Quorum Sensing, Therapeutics.

Introduction

Bacterial biofilms, though microscopic in nature, wield a powerful impact on our health and environment. These intricate microbial communities, cloaked in an extracellular matrix, possess a resilience that challenges conventional treatments. From hospital instruments to the human gut, biofilms thrive in diverse habitats, posing a formidable threat as they resist antibiotics and evade the immune system (Costerton et al, 1999). In this concise overview, we unravel the mysteries of bacterial biofilms, delving into their structure, habitats, and the critical

implications they hold for global health and environmental processes. Join us on this scientific journey to uncover the hidden world of biofilms and their far-reaching consequences.

Building Blocks of Biofilms

Bacterial biofilms are intricate structures formed by microorganisms that secrete an extracellular matrix known as extracellular polymeric substances (EPSs). This matrix, composed of polysaccharides, proteins, lipids, and DNA, acts as a protective shield for the microbial community (Flemming and Wingender, 2010).

Microbial Metropolis

Within extracellular matrix, a bustling city of microorganisms exists, each playing a unique role in the biofilm community. Picture a metropolis where different species or strains collaborate to ensure the survival and growth of the biofilm. Some microbes specialize in nutrient acquisition, while others focus on waste removal or defense mechanisms. It's like a well-oiled machine, where every cog in the system has a vital function (Olaimat et al, 2024).

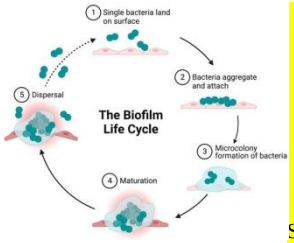
Anchored in Unity

Adhesion is key in the world of biofilms. These communities adhere strongly to surfaces, creating a robust structure that can withstand external pressures. The extracellular matrix acts as the glue that binds the microorganisms together, forming complex three-dimensional architectures. This unity not only ensures the biofilm's survival but also enhances its ability to resist environmental stresses (Santiago et al., 2021).

Slimy Resilience

The slimy nature of the extracellular matrix may seem unassuming, but it plays a critical role in the resilience of biofilms. This slimy shield acts as a barrier, preventing antibiotics and other antimicrobial agents from penetrating the biofilm. It's like a force field that protects the microbial inhabitants, allowing them to persist and flourish in the face of external threats.

Formation of Biofilms



Sharma et al, 2023

Biofilms begin their journey with the initial attachment of microorganisms to a surface, setting the stage for a complex microbial community to emerge. These minute organisms, once tethered, embark on a remarkable transformation, engaging in intricate interactions within the biofilm structure. Through cell communication and the secretion of EPSs, biofilms gradually take shape, evolving into a robust community with a shared purpose (O'Toole et al, 2000).

As more microorganisms join the biofilm, a collaborative effort ensues, leading to the establishment of a mature and resilient structure. The process of biofilm formation involves a choreographed dance of microbial populations, each contributing its unique skills and resources to enhance the overall functionality of the biofilm community. This dynamic interplay fosters the development of specialized roles within the biofilm, enabling efficient nutrient capture, waste removal, and defense mechanisms to safeguard the collective.

Within the intricate matrix of a biofilm, a division of labor emerges, with different microbial species or strains assuming distinct responsibilities. This division of labor not only enhances the efficiency and resilience of the biofilm community but also promotes a harmonious coexistence among its members. As the biofilm thrives and grows in complexity, the collective effort of its inhabitants ensures the survival and adaptability of this resilient microbial ecosystem.

Properties and Functions

Bacterial biofilms possess remarkable properties that contribute to their unique functions and survival strategies. One key characteristic is their strong adhesion to surfaces, enabling biofilms to persist and grow in challenging environments. The extracellular matrix composed of polysaccharides, proteins, lipids, and DNA, provides structural support, allowing biofilm communities to form intricate three-dimensional architectures that enhance their resilience.

Within biofilms, a division of labor emerges as different microbial species or strains take on specialized roles. Some microorganisms focus on nutrient capture, while others excel at waste removal or defense against external threats. This division of labor enhances the efficiency and overall resilience of the biofilm community, allowing for coordinated actions that maximize survival strategies.

Nutrient cycling is a critical function of biofilms, as they engage in complex processes to capture and utilize resources from their environment. This efficient nutrient utilization enables biofilms to thrive in diverse habitats, including extreme environments where traditional microbial communities may struggle. By efficiently recycling nutrients, biofilms contribute to the ecological balance of their surroundings.

The robustness of biofilms is further demonstrated by their enhanced resistance to external stresses, such as antibiotics and immune responses. The extracellular matrix acts as a physical barrier, preventing antimicrobial agents from reaching the microorganisms within the biofilm. This resistance, coupled with the ability to exchange genetic material through horizontal gene transfer, enhances the adaptability and evolutionary success of biofilms in the face of changing environmental conditions.

Habitat and Distribution

Bacterial biofilms exist in a myriad of habitats, both natural and human-made, showcasing their adaptability and resilience. From dental plaque accumulating on our teeth to complex communities thriving in the human gut, biofilms are adept at colonizing various surfaces within our bodies. Their presence extends to industrial settings, where they can clog pipelines and contaminate food processing equipment, causing operational challenges. Additionally, biofilms play a vital role in natural environments, contributing to the ecological balance of aquatic ecosystems by aiding in nutrient cycling and carbon fixation.

The human body serves as a host to diverse biofilm communities, with dental plaque standing as a prime example of their impact on oral health. These biofilms, forming on tooth surfaces, can lead to dental caries and periodontal diseases if left unchecked. Within the human gut, biofilms play essential roles in nutrient absorption, immune modulation, and protection against pathogens, underscoring their significance in maintaining overall health. The intricate

interactions within these gut biofilms highlight the complex nature of microbial communities residing in our bodies.

In industrial settings, biofilms can pose significant challenges by developing on surfaces such as pipelines and water treatment plants. Their growth can lead to corrosion, clogging, and contamination issues, impacting operational efficiency and product quality. Furthermore, biofilms in natural environments, including streams, rivers, lakes, and oceans, contribute to the overall health of aquatic ecosystems through nutrient cycling and carbon fixation processes. Their presence underscores the interconnectedness of microbial communities with the environment.

Understanding the diverse habitats where biofilms thrive is essential for comprehending their ecological roles and potential impact on human health. By delving into the complexities of biofilm distribution, researchers can uncover new insights into how these microbial communities interact with their surroundings and the implications they hold for both natural ecosystems and human well-being. The study of biofilms in various habitats opens up avenues for innovative research and sustainable practices that can help mitigate their adverse effects and harness their beneficial properties.

Common Biofilm Forming Microbes

Common biofilm-forming bacteria include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus mutans*. These bacteria can form biofilms on medical devices, human tissues, and industrial surfaces, leading to persistent infections and contamination. The biofilm mode of growth is a significant challenge in healthcare and industry due to its role in chronic infections and its contribution to the difficulty in eradicating bacterial contaminants.

Challenges in Healthcare Settings

Biofilms pose a significant challenge in healthcare settings, especially in the context of medical device-related infections. These resilient microbial communities can form on catheters, implants, and surgical instruments, leading to persistent and difficult-to-treat infections. The ability of biofilms to evade the immune system and resist antibiotic treatments complicates patient care and prolongs recovery times. Healthcare professionals face an ongoing battle to

effectively manage and eradicate biofilm-related infections, which can result in increased morbidity, mortality, and healthcare costs.

Chronic Infections and Treatment Resistance

One of the most pressing implications of biofilms for human health is their role in chronic infections. Biofilm-associated infections are notoriously difficult to eradicate, as the extracellular matrix provides a protective shield for the embedded microorganisms. This resistance to conventional antimicrobial therapies poses a serious threat to patient outcomes and public health. Finding innovative strategies to disrupt biofilm formation and enhance the effectiveness of treatment regimens is crucial in combating the growing problem of antibiotic resistance and treatment failure (Hall and Stoodley, 2009).

Medical Device-Related Infections

Biofilms are commonly implicated in medical device-related infections, such as catheter-associated urinary tract infections and surgical site infections (Stewart and Costerton, 2001). The presence of biofilms on medical implants and devices not only compromises their functionality but also increases the risk of systemic infections (Patel, 2005)**.** Healthcare providers must remain vigilant in preventing and managing biofilm-related infections, implementing stringent infection control measures and exploring new technologies to mitigate the risk of device-associated complications **(**Donlan, 2002).

Immune System Evasion

One of the key characteristics of biofilms is their ability to evade the host immune system. The extracellular matrix acts as a physical barrier, shielding the microbial community from immune cells and antibodies. This immune evasion mechanism allows biofilms to persist and thrive within the human body, leading to chronic and recurrent infections. Understanding the complex interplay between biofilms and the immune response is essential for developing targeted immunotherapies and enhancing the host's ability to combat biofilm infections.

Public Health Impact

The prevalence of biofilm-related infections in healthcare settings has a profound impact on public health. The rise of antibiotic-resistant biofilm-forming pathogens poses a serious threat

to global health security, necessitating a coordinated and multidisciplinary approach to combating these challenging infections (Mah and O'Toole, 2001). Public health initiatives aimed at raising awareness about the risks of biofilms, promoting infection prevention strategies, and fostering research collaborations are essential for addressing the growing burden of biofilm-associated diseases.

Environmental Significance

Bacterial biofilms not only impact human health but also play a vital role in environmental processes. These microbial communities are key players in bioremediation, aiding in the breakdown and detoxification of waste and toxic substances. By harnessing the capabilities of biofilms, we can promote sustainable environmental management practices and contribute to a healthier ecosystem.

In natural habitats like streams, rivers, lakes, and oceans, biofilms contribute to nutrient cycling and carbon fixation, maintaining the ecological balance of aquatic ecosystems. Their presence ensures the efficient breakdown of organic pollutants and the removal of heavy metals, thereby preserving the purity of our water sources. This essential role highlights the significance of biofilms in maintaining environmental health.

Within industrial settings, biofilms can form on surfaces such as pipelines, water treatment plants, and food processing equipment. While they may lead to operational inefficiencies and product quality concerns, biofilms also have the potential to aid in the treatment of wastewater and the degradation of contaminants. Understanding and leveraging the environmental benefits of biofilms can lead to innovative solutions for pollution control and resource conservation.

As we delve deeper into the world of bacterial biofilms, it becomes evident that their impact extends beyond the confines of human health. By recognizing and appreciating the environmental significance of biofilms, we can work towards a more sustainable future where these microbial communities play a crucial role in maintaining the balance of our ecosystems. Through continued research and innovative approaches, we can unlock the full potential of biofilms in environmental stewardship.

Horizontal Gene Transfer in Biofilms

Biofilms are thought to be hot spots for horizontal gene transfer (HGT) of antibiotic resistance genes (ARGs). ARGs can be spread via HGT, though mechanisms are known and have been shown to depend on the environment, bacterial communities and mobile genetic elements. Classically, HGT mechanisms include conjugation, transformation and transduction; more recently, membrane vesicles (MVs) have been reported as DNA reservoirs implicated in interspecies HGT (Abe et al. 2020).

Emerging Therapeutic Strategies

Biofilm-related diseases, caused by microbial communities that adhere to surfaces in the body, present significant therapeutic challenges due to the increased resistance to conventional antibiotics and the complexity of biofilm structures. Recent research has led to emerging therapeutic strategies aimed at disrupting biofilms or preventing their formation. (Mishra et al, 2023). Below are some of the most promising approaches:

1. Anti-biofilm Agents

Enzymatic Disruption: Enzymes like DNase I, proteases and dispersin B can break down the extracellular matrix of biofilms, which is often composed of DNA, proteins and polysaccharides. By targeting these structural components, enzymes help to weaken the biofilm and enhance the efficacy of antibiotics. **Example**: DNase I is used to degrade extracellular DNA, which is a key component of biofilms, particularly in *Pseudomonas aeruginosa* infections.

Antimicrobial Peptides: Almost every organism produces antimicrobial peptides (AMP), sometimes referred to as host defense peptides (HDP), which are conserved antimicrobial compounds. These peptides are cationic because they include an excess of lysine and arginine residues and are made up of 12–50 amino acids. Additionally, they interact with bacterial membranes because they are highly hydrophobic, which increases their antibacterial effectiveness. While certain AMPs have immune-modulating properties without potent direct antimicrobial activities, the majority of AMPs exhibit direct antimicrobial activity by rupturing bacterial membranes. It has recently been demonstrated that a range of synthetic and natural peptides have unique antibiofilm activity against both Gram-positive and Gram-negative bacteria. Given their small size, low production costs, low toxicity, relative stability, and

specificity for biofilms at lower concentrations than the minimum inhibitory concentration (MIC) for planktonic cells, synthetic antimicrobial peptides may be viable options for treating biofilms (Bernardes *et al*, 2015).

2. Phage Therapy

Bacteriophage-based Therapies: Bacteriophages, or phages, are viruses that infect bacteria and can be engineered to target biofilm-forming pathogens. Some phages produce depolymerases that degrade the biofilm matrix, making bacteria more susceptible to antibiotics. Example: Phage therapy has been used experimentally to treat chronic infections caused by *Staphylococcus aureus* and *Pseudomonas* species.

3. Nanotechnology

Nanoparticles and Nano-molecules: Nanoparticles, such as silver nanoparticles and nanomaterials, can penetrate biofilm structures, disrupting the microbial cells and the biofilm matrix. Some nanoparticles also have antimicrobial properties. Example: Silver nanoparticles have shown effectiveness in preventing biofilm formation by various pathogens, including *E. coli* and *Staphylococcus aureus*.

Nanostructured Surfaces: Biofilm formation can be reduced by designing materials with surfaces that either prevent bacterial adhesion or actively repel microbial attachment, often using nanotechnology.

4. Anti-virulence Strategies

Quorum Sensing Inhibitors: Many biofilm-forming bacteria use quorum sensing to communicate and coordinate biofilm formation. Inhibiting these signaling pathways can prevent the development of biofilms without killing the bacteria, reducing the likelihood of resistance development. Example: Disrupting the quorum sensing in *Pseudomonas aeruginosa* using synthetic inhibitors or small molecules can prevent biofilm formation in respiratory infections. Some of the common quorum sensing inhibitors are: Flavonoids, Terpenoids, Phenols etc.

5. Combination Therapies

Antibiotic Combinations: Combining traditional antibiotics with biofilm-disrupting agents, such as enzymes or nanoparticles, can be more effective than using antibiotics alone. This dual

approach targets both the bacterial cells and the biofilm matrix, increasing treatment efficacy. Example: A combination of rifampin and DNase I has been found to enhance the treatment of biofilm-associated infections.

6. Photodynamic Therapy (PDT)

Light-activated Therapy: PDT uses photosensitizers that, when activated by light, generate reactive oxygen species (ROS) that damage both the biofilm and bacteria. This is a promising approach for infections in areas that can be exposed to light, like dental implants and chronic wounds. Example: PDT has been shown to be effective in reducing biofilm formation in oral pathogens like *Streptococcus mutans* and *Porphyromonas gingivalis* (*de Melo et al, 2013).*

7. Gene Editing and RNA-based Therapies

CRISPR-Cas and RNA Interference: Gene editing tools like CRISPR-Cas9 are being explored to disrupt genes responsible for biofilm formation. Similarly, RNA interference (RNAi) could be used to silence genes related to biofilm production, preventing bacterial adhesion and biofilm development. Example: Studies are underway to target biofilm-related genes in *Staphylococcus aureus* to reduce biofilm formation in chronic infections (Ghosh et al, 2022).

8. Immunotherapy

Immune Modulation: Harnessing the host's immune system to fight biofilms is another innovative approach. For example, antibodies or immune modulators could be designed to target biofilm components directly, aiding in their clearance. Example: Antibodies targeting biofilm components or bacterial surface proteins have shown promise in reducing biofilm formation in animal models (Shrestha et al, 2022).

9. Natural Product-Based Therapies

Plant-derived and Natural Compounds: Several natural products, including essential oils, peptides, and flavonoids, have demonstrated anti-biofilm properties. These compounds can interfere with biofilm formation and disrupt existing biofilms. Example: Compounds derived from garlic, curcumin, and tea tree oil have been found to reduce biofilm formation in both Gram-positive and Gram-negative bacteria (Lu *et al.* 2019).

10. Surface Modification of Medical Devices

Coating and Surface Engineering: Medical devices, such as catheters and implants, are often associated with biofilm-related infections. Coating these devices with anti-microbial or anti-biofilm agents (e.g., antibiotics, silver, or polymer coatings) can reduce bacterial adhesion and biofilm formation. Example: Silver-coated catheters and devices are used to prevent biofilm-related infections in hospitals (Donlan, 2001).

11. Inhibition of Biofilm Formation by Pilicides and Curlicides

Most bacterial infections need bacterial attachment, which is the first stage in the biofilm building process. Bacteria have developed intricate pili and fimbriae systems to enable adhesion to the epithelial surface. Adhesin, tip fibrillium, adaptor subunits, pilus base, termination, and anchoring units are among the components that make up the extremely conserved pili structure. Some bacteria, such as E. coli and other Enterobacteriaceae, create curli in addition to pili. These curli are sticky amyloid fibers that are found on the surface of bacterial cells and are known to be essential for the formation of biofilms. Consequently, pili and curli are ideal targets for regulating bacterial adherence and biofilm formation. These Pilicide's and Curlicide's capacity to decrease the development of fimbriae and the swarming motility of bacteria—likely by interfering with curli and/or pili—was thought to account for their antibiotic effectiveness. All of these results point to the possibility of using curlicides and pilicides as a therapeutic approach to treat a number of biofilm infections, either on the surface of implants or in dental hygiene products (Krukiewicz *et al*, 2022).

12. Antibacterial/Anti-Adhesive Porous Oxide Layers

Plasma electrolytic oxidation (PEO) is widely used to form porous oxide layers on various metal surfaces, including titanium, aluminum, magnesium, niobium, tantalum, and their alloys. These materials have been found to be applicable to the design of short-term or long-term bone implants. As a result of the plasma electrolytic process, a porous layer is formed on the surface of the metal, with pores in the range of several nanometers to several micrometers. The pore size strongly depends on the parameters applied during the anodization process, such as the voltage, current density, bath and chemical composition of the treated metals, and duration of the process. The surface pretreatment (e.g., grinding, polishing, sand-blasting, or etching) also influences the final porous oxide layer. Microstructures on the oxide layer are favorable for bone tissue

formation; therefore, this process has potential applicability as a surface treatment for long-term implants (Leśniak *et al*, 2021).

Obstacles in the Development of Therapeutic Strategies

Microbes, EPS and the surface interact dynamically and intricately during the biofilm development process. Microbes' adhesion strength and visco-elastic characteristics help them build biofilms and make them resistant to antimicrobial substances. One major problem with treating biofilms is that using antimicrobials alone frequently leaves behind minute biofilm and cell debris residues. Although cells may survive in these residues, they will also probably make it easier for microorganisms to colonize the area in the future. For example, if treatment is stopped or after cells have become resistant, they may become virulent once more. The aforementioned results have led to the conclusion that the treatment plan ought to focus on both EPS and the remaining microbes (Asma *et al.* 2022).

Future Research Aspects

Future research on biofilm-related diseases focus on developing more effective strategies to combat the persistent and resilient nature of biofilms. The complexity of biofilm formation, their resistance to antibiotics and the challenges in treating chronic infections are crucial. Below are some key research aspects that will shape the future of biofilm-related disease management:

1. Understanding Biofilm Formation Mechanisms

Molecular Pathways and Signaling: Biofilm formation is a highly regulated process involving genetic, metabolic and environmental factors. Future research will focus on uncovering the molecular mechanisms that regulate biofilm development, such as quorum sensing, genetic regulatory networks and environmental cues (e.g., nutrient availability, pH and oxygen tension). A deeper understanding of these processes could lead to the development of targeted therapies that disrupt biofilm formation at the genetic or signaling level (Camilli and Bassler, 2006).

Interfering with c-di-GMP Signaling : Bis-(3′-5′)-cyclic dimeric guanosine monophosphate (c-di-GMP) is a bacterial second messenger molecule that governs the motility, virulence and cell cycle of some gram-negative and gram-positive bacteria. It is known that high levels of c-di-GMP enhance bacterial adhesion by reducing the expression or activity of flagella and stimulating the production of bacterial adhesives and EPS. Therefore, controlling c-di-GMP

metabolism could be an efficient way to modulate the formation of biofilm structures (Purcell and Tamayo, 2016).

Host-Biofilm Interactions: Research into the interactions between biofilms and the host immune system is crucial. Understanding how biofilms evade the immune response or manipulate host cells could reveal potential therapeutic targets to enhance immune clearance of biofilm infections. (Chen and Kolodkin, 2022).

2. New Diagnostic Tools

Early Detection: Early diagnosis of biofilm-associated infections remains a challenge. Research is focused on developing non-invasive and rapid diagnostic methods to identify biofilms in clinical settings, including imaging techniques (e.g., advanced microscopy, fluorescence, and confocal laser scanning) and biosensors.

Point-of-Care Diagnostics: Portable, easy-to-use diagnostic devices that can detect biofilm-related infections at the point of care could revolutionize the management of chronic infections. Research into biosensors or lab-on-a-chip technologies for biofilm detection is an important emerging field (Wang et al, 2024).

3. Polymicrobial Biofilms

Understanding Mixed Species Biofilms: Many chronic infections involve polymicrobial biofilms, where multiple bacterial species coexist and interact. Future research will focus on understanding how these mixed biofilms form, interact, and develop resistance to treatment. This will help develop strategies that target multiple species in a single biofilm (Biswas et al, 2024).

Targeting Inter-species Communication: Research into interspecies interactions within polymicrobial biofilms and how they communicate could provide new therapeutic targets. This research could lead to strategies that prevent cooperation between different bacterial species, thus disrupting biofilm stability (Parashar et al, 2015).

The future of biofilm-related disease research lies in a multi-faceted approach, combining a deeper understanding of biofilm biology with novel therapeutics, advanced diagnostic methods, and personalized medicine. By addressing the unique challenges posed by biofilms, future

research holds promise for developing more effective treatments for chronic and implant-associated infections, while reducing the risk of antibiotic resistance.

Conclusion

In conclusion, bacterial biofilms are a fascinating and complex aspect of microbial ecology that play a significant role in human health and environmental processes. The formation of bacterial biofilms and the bacterial resistance to antibiotics have been recognized as critical challenges facing modern medicine. These problems are further complicated by the emergence of multidrug-resistant pathogens whose eradication now requires the use of last-resort antibiotics. Their intricate structure, ability to form in various habitats and resistance to conventional treatments make them a formidable challenge to overcome. Understanding the properties and functions of biofilms is crucial for developing new strategies to combat their negative impacts on human health, such as in hospital-acquired infections, and on the environment, such as in biofouling of marine ecosystems. In addition to antimicrobial chemotherapy, other strategies include the use of compounds that degrade the matrix, inhibit cell-to-cell signaling, increase susceptibility of the biofilms to antimicrobial compounds and phagocytosis, or in the case of implants, remove/replace contaminated implants or change their physicochemical properties. Current research efforts are focused on developing nanostructured surfaces with adhered/incorporated or melted anti-adhesive agents. It is anticipated that the number of studies related to the production of novel materials destined for medicine should and will increase.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

Ethics Approval Statement

Not Applicable

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Data Availability Statement

Not Applicable

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Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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