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RESEARCH ARTICLE

BIOFILM OF STAPHYLOCOCCUS AUREUS ON DIFFERENT DENTURES MATERIALS

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Abstract

A common opportunistic infection, *Staphylococcus aureus*, is remarkably adept at forming biofilms on abiotic and biological surfaces, including several kinds of dental prosthesis materials. Antimicrobial resistance, systemic infections, and denture stomatitis are all seriously threatened by the development of biofilm on denture surfaces in the oral environment. The prevalence of denture stomatitis has significantly increased in recent years, especially among denture users. An erythematous reaction on the oral mucosa that spreads to the boundaries of the maxillary denture bearing area is a frequent inflammatory reaction with a multifactorial origin that typically occurs in the oral cavity. Medical extracts with antibacterial action against oral pathogens have recently been presented as a less harmful and safer treatment option for denture stomatitis. It is critical to reduce the risk of both local and systemic infections in immunocompromised cancer patients with maxillary abnormalities, according to the findings of this literature study. Determining how saliva affects microbial adherence to obturator materials is also crucial, as is creating materials with longer lifespans and surface properties that encourage less microbial attachment than existing materials. Highlighting several facets of *S. aureus* biofilm development and its overall architecture, as well as its constituent parts, clinical consequences, and involvement in pathogenesis and drug resistance, is the aim of this review. Along with discussing numerous tactics that can be utilized to inhibit and eradicate *S. aureus* biofilm, the review also covers the many methodologies used in the qualitative and quantitative investigation of *S. aureus* biofilm.

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Introduction:-

Staphylococcus aureus

is a Gram-positive opportunistic pathogen that mostly colonizes the skin and mucous membranes. It is thought to be asymptomatic in 30% of healthy persons. It is a leading cause of infections of the skin and soft tissues, especially in those who are already colonized. According to recent international surveys, *S. aureus* is the main infection that kills people over the age of 15 and the major bacterial cause of death in 135 countries. *S. aureus*-caused superficial skin

infections frequently resolve on their own, but they can serve as gateways for the bacteria to enter deeper tissues and the circulation, which could lead to potentially fatal systemic infections(1).

One of the most prevalent harmful bacteria that infects hospitalized individuals is *S. aureus*. The worldwide healthcare system is under strain due to the high death and recurrence rates of invasive *S. aureus* infections. Another well-known characteristic of *S. aureus* is its capacity to develop and transmit multiple antibiotic resistance (AMR) (2).

Denture users are more likely than non-wearers to have *Staphylococcus aureus* in their oral natural flora. Dental prosthesis comprise one of the various surfaces in the oral cavity that *S. aureus* can attach to. Staphylococcal biofilm can particularly easily colonize dentures, which are non-shedding oral surfaces. Numerous infections can be caused by *S. aureus*. These include infections related to the buccal region, particularly angular cheilitis, periodontitis, mucositis, and infections linked to dental implants. The majority of harmful side effects might happen when an infectious agent enters the body from the respiratory system and develops pneumonia. (3). In the past, bacteriologists believed that *Staphylococcus aureus* colonization of the oral cavity had no effect on the health of the mouth. Nonetheless, evidence over the last 10 years indicates that *S. aureus* inhabits the oral cavity more frequently than initially thought (more frequently than the nasal vestibule) (4). and that could lead to an assortment of systemic and oral illnesses. Within a sessile community called a biofilm, *Staphylococcus aureus* methods an extracellular polymeric substance (EPS) that aids in germ resistance or lessens the antibacterial effect.

These results demonstrate how *S. aureus* has evolved dynamically through mobile genetic components and emphasize the need for uniform metadata in public genomic databases to enhance surveillance. They also highlight how important it is to use a One Health approach when tracking the evolution of *S. aureus*, especially when it comes to the co-dissemination of resistance and biofilm genes across different ecological niches (5).

Biofilm formation in *Staphylococcus aureus* begins with the attachment of free-floating planktonic cells to a suitable surface, initiating colonization. The adherence of *S. aureus* to a surface is governed by physicochemical interactions, particularly hydrophobic and hydrophilic forces between the bacterial cell surface and the substrate. Studies have shown that *S. aureus* adheres more readily to hydrophobic surfaces through numerous weakly binding macromolecules, whereas adhesion to hydrophilic surfaces typically involves fewer, but stronger, molecular interactions. These initial attachment mechanisms are critical for the establishment and stability of biofilms on both biotic and abiotic surfaces(6).

The *S. aureus* cell surface has been shown to cling to hydrophilic surfaces with fewer but stronger binding macromolecules, whereas it adheres to hydrophobic surfaces with the aid of numerous weakly binding macromolecules. Following the development of microcolonies, an extracellular polymeric substance (EPS) forms and eventually matures into a biofilm. The bacterial cells that live inside the biofilm emit certain compounds, such as D-amino acids and EPS-degrading enzymes like alginate lyase, to break and disseminate the biofilm once it has fully grown (7).

concept of biofilm

A completely regular group of microorganisms embedded in a certain matrix was described using the term "biofilm." This biofilm can adhere to both inanimate and living surfaces. Oral infections, particularly denture stomatitis, may be primarily caused by biofilm growth on the denture base. Between 30% and 75% of people who wear dentures are afflicted by this illness. In the palatal mucosa that comes into direct touch with the fitting surface of a partial or full prosthesis, it manifests as erythema. (8) Because biofilms are difficult to diagnose and lack indicators, they are especially difficult to cure.

Because biofilm communities are complex and antibiotic-resistant, new material science is needed to identify and apply solutions, especially for biofilm-resistant materials and traditional antibiotics. Targeting bacterial functions like quorum sensing, biofilm-related gene expression, secondary messengers, and regulatory RNA, as well as preventing initial adhesions with green technology like silicon oil-infused substrates from plant models, are examples of sustainable innovations in antifouling that are being investigated (9).

Biofilm composition

The two main components of *S. aureus* biofilm are water (about 97%) and organic matter, which includes microcolonies and EPS (10). Because of its chemical makeup, polysaccharide-intercellular-adhesin (PIA), also known as poly-(1-6)-N-acetylglucosamine (PNAG), is the main constituent of *S. aureus* biofilm EPS. Because of its positive charge, PIA promotes colonization, biofilm formation and biofilm-based infections, immune system evasion, resistance to antibiotics, and phagocytosis (3).

Biofilms and Microbial Adhesion

A biofilm is a three-dimensional matrix formed when bacteria adhere to a surface by releasing gelatinous exopolymers that are mainly insoluble. A collection of extracellular materials and bacteria on a solid surface is called a biofilm. From a medical perspective, biofilm-like clusters formed by both beneficial and dangerous microorganisms can attach to the surfaces of teeth or medical implants, become embedded in the mucous layer of the bowels, lungs, or vagina, or be linked to an epithelial or endothelial lining (11). Because microorganisms forming as biofilms are less vulnerable to topical treatments, antibiotics, and host defenses than are planktonic versions of the same microorganisms, biofilm formation and persistence have consequences for the patient. A lot of biofilm infections are hard to cure and frequently show up as persistent or recurring infections.

Numerous clinical issues are brought on by biofilm infections, such as illnesses involving nonculturable species, persistent inflammation, poor wound healing, quickly developing antibiotic resistance (12). Microorganisms cling securely to a surface through physicochemical interactions known as adhesion, which include a time-dependent phase of irreversible chemical and cellular adhesion and an initial period of reversible physical contact. For the microbes and surfaces to establish an adhesive connection, energy in the form of electrostatic, hydrophobic, and/or van der Waals forces is needed. Bacterial adhesion (the first process of bacteria attaching directly to a surface) is more commonly referred to as adherence.

The first stage of bacterial adhesion is called attachment, which is typically reversible and refers more to physical contact than intricate chemical and cellular interactions (13). Microbial adhesion can be impacted by general environmental factors such temperature, exposure duration, microbial concentration, and the presence of antibiotics. For instance, the quantity of bacteria that stick to substrata surfaces grows over time until a saturation level unique to each kind of surface is attained. By altering physical interactions or the surface properties of the bacteria or materials, these factors can affect bacterial adherence (14).

Microbes strategy through the formation of biofilms

By forming biofilms, microbes have developed a special survival strategy. Multiple microorganisms transition from the planktonic state to develop intricate matrix-like structures called biofilms by combining together as "communities." Dense micro-communities known as biofilms form on inert surfaces and encase themselves in secreted polymers. By changing their patterns of gene expression, organisms that create a biofilm can adjust to changes in their environment. The microorganisms can be shielded from antibiotics or disinfectants by the biofilm formation and associated changes in gene expression. A major public health hazard may arise from the resultant biofilm (15).

Denture-Associated Biofilm Microbiota

A completely regular group of microorganisms embedded in a certain matrix was described using the term "biofilm." This biofilm can adhere to both living and nonliving surfaces (16). Oral infections, particularly denture stomatitis, may be primarily caused by biofilm growth on the denture base. Between 30% and 75% of people who wear dentures are afflicted by this illness. In the palatal mucosa that comes into direct touch with the fitting surface of a partial or full prosthesis, it manifests as erythema.

Factors Influencing Biofilm Development

- Surface roughness: Greater roughness increases microbial retention and biofilm biomass.
- Hydrophobicity: Hydrophobic interactions promote adhesion of *S. aureus* to certain materials.
- Salivary pellicle formation: Salivary proteins can enhance or reduce adhesion depending on their composition.
- Material aging and wear: Long-term use alters surface topography and increases susceptibility.

Epidemics, food spoiling, and equipment damage are all thought to be caused by biofilms. Therefore, it is essential to have a thorough understanding of all the elements that influence the growth or development of bacteria, such as the attachment surface, surrounding circumstances, related bacterial cells, and surface electrostatic charging. Environmental cues and elements of the bacterial extracellular surface are essential for biofilm development and autoaggregation. [Proteinaceous features including pili and fimbriae, lipopolysaccharides, and outer membrane proteins are known to affect the phenotype of bacterial adhesion and autoaggregation because of their advantageous positions on the cell surface show fig (1) (17)

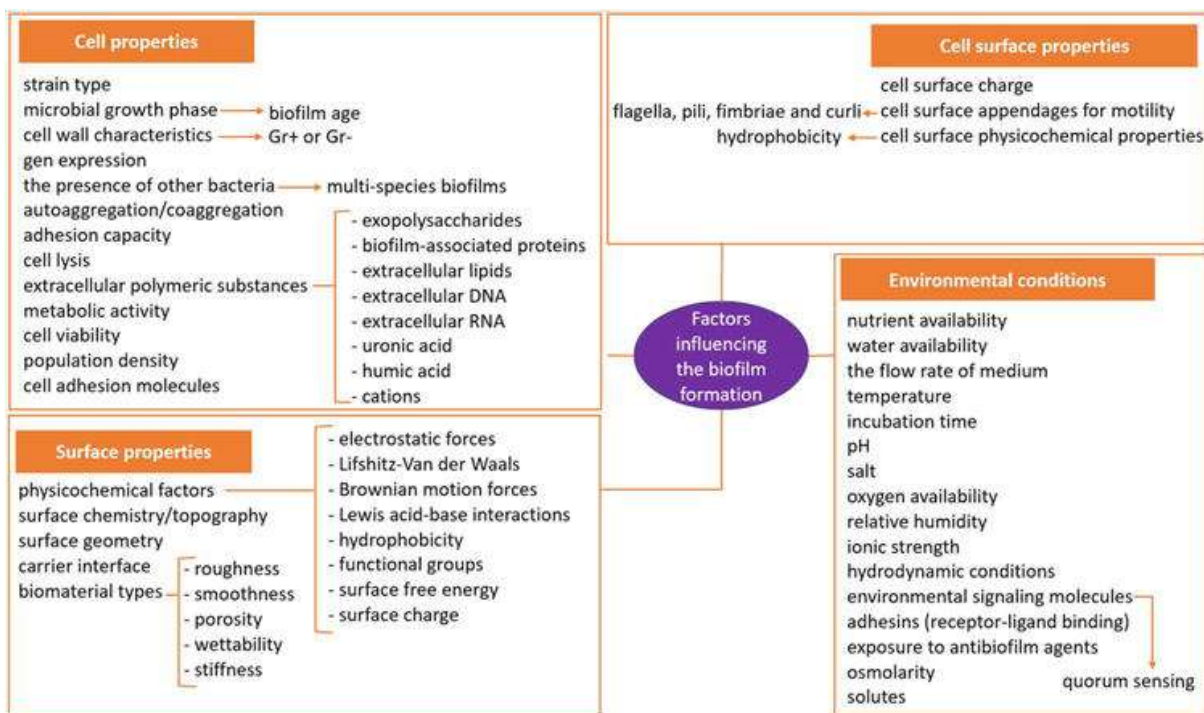


Figure 1. Factors affecting biofilm formation (18)

The majority of the time, microorganisms stick to surfaces quickly. Initial attachment in the biofilm life cycle happens quickly through physicochemical interactions between the bacteria and the surface. Gene expression shifts quickly, and as the EPS physically affixes cells to the surface, biofilm structure formation starts. Furthermore, both electrostatic and non-electrostatic interactions between the bacteria and the solid surface cause the physicochemical phenomena known as nonspecific adherence of microorganisms to surfaces. Electrostatic forces are produced between the surface of the microbe and the receiving surface when electrostatic double layers joined by charged groups on either surface come into contact. Material Surface Characteristics(19)

The attachment to the surface could be temporary or permanent, depending on the kind of interaction. Bacterial cells may use surface adhesins to form an irreversible surface attachment under environmental conditions. Adhesion to biotic surfaces usually necessitates a specific receptor-adhesin connection, whereas adhesion to abiotic surfaces is frequently mediated by nonspecific interactions (20)

The factors that influence microbial adherence to a biomaterial surface comprise the material's chemical composition, surface charge and hydrophobicity, and surface roughness or physical configuration. The formation of biofilms and the adsorption or binding of salivary proteins may impact the surface's hydrophobic and hydrophilic characteristics, surface energy, and the availability of empty binding sites (21)

Acrylic Resin (Polymethyl methacrylate, PMMA)

For instance, *S. aureus* preferentially sticks to metals while *Staphylococcus epidermidis* preferentially sticks to polymers. This could help explain why *S. aureus* is frequently the main pathogen in infections caused by implanted

metal medical devices, but *S. epidermidis* is frequently linked to infections caused by implanted polymeric medical devices. Bacterial adherence to surfaces that have been altered or modified with a coating, such as a nonsteroidal anti-inflammatory drug coating, an antimicrobial peptide coating, or a pluronic surfactant coating, is prevented(22) Most commonly used denture base material.

- Its porous surface and surface roughness promote microbial adhesion and biofilm maturation.
- *S. aureus* forms robust biofilms on untreated PMMA due to its hydrophobic nature and micro-porosities

Metal Alloys (e.g., Cobalt-Chromium)

Surface roughness is a three-dimensional characteristic of a material's surface, typically quantified using roughness-measuring systems such as the stylus profilometer. It is commonly expressed as arithmetic average roughness (R_a), which represents the average vertical distance between peaks and valleys on the surface. Terms like surface finish and surface smoothness are often used interchangeably(23).

Numerous studies have shown that surface roughness significantly influences microbial adhesion and subsequent biofilm formation. This relationship can be attributed to several factors:

1. Initial bacterial adhesion tends to occur in surface irregularities where microorganisms are shielded from shear forces, allowing the transition from reversible to irreversible attachment.
2. Increased surface area due to roughness provides more available sites for microbial attachment—often 2 to 3 times greater than smoother surfaces.
3. Cleaning challenges associated with rough surfaces allow residual cells to remain, facilitating rapid biofilm regrowth through cell multiplication rather than recolonization.

Bacterial adherence and retention are similarly influenced by surface free energy. Higher energy surfaces have a tendency to draw in more plaque, bind it more firmly, and possibly even favor particular bacterial species. Nevertheless, a number of studies have shown that surface roughness might have a greater impact than surface free energy. For example, wear and other degradation processes, as well as hydrolysis of the silane interface between the polymer matrix and inorganic filler particles, cause composite fillings to have a rougher surface. Enhanced plaque buildup, especially in older composite resin dental restorations, is more likely to be explained by this increasing roughness than by modifications in the physicochemical surface characteristics (24).

Thermoplastic Resins (e.g., Nylon-based flexible dentures)

A dental device called a removable partial denture (RPD) is used to replace lost teeth in order to enhance masticatory efficiency, improve phonetics, and prevent undesired tooth movement. Given the growing number of adults who are partially dentate, the percentage of people who wear partial dentures is rising. This increase most likely reflects a transition from complete to partial edentulism as oral hygiene improves, as well as an increase in the population's average age and life expectancy(25). The oral cavity is a dynamic environment, so any prosthetic material used to replace lost teeth should have certain properties. The denture base is the part of the RPD that shields the oral cavity's soft tissue and supports the prosthetic teeth. Metal or acrylic make up the majority of denture base materials (DBMs). However, each has restrictions of its own. Polymer-based DBMs are more prone to fracture than metallic DBMs, which are hefty and technique-sensitive during manufacture.(26)

Strength, durability, processing accuracy, dimensional stability, acceptable thermal characteristics, biocompatibility, high insolubility and low sorption in oral fluids, chemical stability, superior aesthetics, ease of manufacture and cleaning, and other qualities are all necessary for the perfect DBM. It should also stick well to relining material and artificial teeth. It should be biocompatible with the soft tissues of the mouth in terms of biological characteristics.

Lastly, it should be inexpensive and simple to fix (27)

Show variable biofilm formation depending on their composition and surface finish. Their flexibility may lead to micro-movements that encourage microbial colonization in crevices. For removable partial dentures (RPDs) to be successful, the material qualities used to manufacture the denture base material must be carefully considered. Nylon denture bases are a popular substitute for polymethyl methacrylate (PMMA) in RPDs due to their flexibility. Because they form a seal around the denture's edge, flexible dentures aid in retention. In this paper, we summarize the applications, benefits, and drawbacks of flexible dentures based on the most recent research (28)

3D-Printed Denture Resins

- Emerging materials with potential for improved smoothness and reduced porosity.
- However, depending on the printing method and post-processing, surface irregularities may still support biofilm formation.

Complete dentures continue to be the preferred treatment for edentulous patients whose alternatives for treatment are restricted because of systemic illnesses, oral health issues, or financial limitations. To guarantee longterm durability and patient satisfaction, denture base resin (DBR), the main component of complete dentures, needs to have exceptional mechanical strength, stability, and biocompatibility. Anodized nanosurfaces of medical implants with improved osseointegration and decreased polymerization can be produced by combining 3D with nanoparticles, therefore enhancing biocompatibility, durability, and cost effectiveness.

The possible beneficial antimicrobial effects of using 3D technology and nanoengineering in dental and orthodontic implants, oral prostheses, joint replacements, hearing aids, catheters, stents, endotracheal tubes, prosthetics, and bone scaffolds are examined in this research (29). A new industry of medical equipment that are safer to use without worrying about post-operative infections has emerged as a result of the use of 3D-printed reusable medical devices in fields other than dentistry. In the medical field and the healthcare sector, persistent infections brought on by bacterial biofilm formation on implanted medical devices are a serious concern.

Human infections can be caused by a variety of pathogens, including bacteria, viruses, and fungi, but bacterial infections are the most prevalent kind, accounting for both acute and chronic infections in the general population (30). There are two types of bacteria: sessile, which sticks to the surface, and planktonic, which floats freely. A protective barrier that functions as an endogenous defense mechanism is produced in both phases, making it more difficult for antibiotics to eradicate the infection-causing bacteria. Together with the accumulated microbial cell community, this exopolysaccharide matrix barrier, also known as "slime," is what is today called "biofilm" (31).

Zirconia and Ceramic Materials

Generally exhibit low *S. aureus* adherence due to high surface hardness and low porosity. They are more resistant to microbial colonization but are less commonly used for full dentures. The influence of the electrostatic state and its connection to bacterial adherence were not sufficiently demonstrated by the data obtained for dental ceramics. Nonetheless, research presented in this review indicates a relationship between topography, surface free energy, and bacterial adhesion (32). As a biocompatible, aesthetically pleasing, and long-lasting substitute for conventional titanium implants, zirconia dental implants have become a game-changer in the field of implantology.

The main characteristics of zirconia, including as its low bacterial affinity, superior aesthetics, and great fracture resistance, are examined in this thorough overview. Because zirconia can osseointegrate with bone and is resistant to inflammation and plaque, it makes a product that is especially well-suited for patients who have high aesthetic standards or metal sensitivity. But problems like brittleness and intricate manufacturing procedures still exist. These restrictions are about to be addressed by developments in surface modification methods and material optimization, opening the door for more widespread uses (33).

Because of its improved biocompatibility, aesthetic benefits, and resistance to corrosion and the production of bacterial biofilms, zirconia dental implants have become a viable substitute for titanium implants. Zirconia is a great option for people who are sensitive to metals or who are at risk of developing peri-implantitis because studies have repeatedly shown that it can lower inflammation and promote improved peri-implant health. The cosmetic requirements of contemporary dentistry are also met by its natural tooth-like look, especially in the anterior region. Furthermore, zirconia's lower heat conductivity and corrosion resistance reinforce its potential as a long-lasting and patient-friendly material (34). Biocompatibility:

Zirconia interacts well with human tissues since it is very biocompatible. It is appropriate for people with metal sensitivities or allergies since it reduces the possibility of negative reactions, inflammation, or rejection. Research has demonstrated its capacity to sustain healthy peri-implant tissues and encourage soft tissue recovery. Research has indicated that there are no appreciable variations in bone-to-implant contact and removal torque values when compared to titanium implants. In fact, acid-etched zirconia implants were found to have significantly higher BIC values than titanium implants, highlighting their remarkable bioactivity, chemical stability, and reduced inflammatory response.

Zirconia Implant Types: Zirconia dental implants come in a variety of forms, each intended to satisfy certain patient requirements and clinical preferences. The intended use, material composition, and design of these implants differ. (36)

Conclusions:-

This study supported the idea that the evolution of *S. aureus* clonal complexes already known for their great virulence and resistance is very dynamic by identifying important characteristics including biofilm generation and resistance genes that suggest possible co-dissemination. The bias in the NCBI public databases, which primarily represent *S. aureus* in the clinical settings of wealthier nations and continents, was another important discovery. This literature review leads to the conclusion that numerous recent research have investigated the role of multi-species biofilms in the emergency of denture stomatitis.

Therefore, it is now essential to explain biofilm adhesion to various surfaces and how to prevent it. The intricacy of biofilm is caused by highly regulated gene expression networks and cell-cell interactions. Understanding how gene expression changes in tandem with biofilm formation on denture and catheter surfaces enables the use of these simulations to verify potential biofilm growth inhibitors. Given that certain plant extracts have antibacterial properties, these medicinal extracts should receive a lot of attention and additional research to assess their inherent antiplaque properties. The significant usefulness of this new field of biofilm targeting is greatly increased by the prospect of even greater breakthroughs in the future when additional chemicals and faster printing techniques are found.

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Data availability in this published article (and its supplementary information file), all data generated or analyzed during this study are included.

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