HSR London International Conference on Multidisciplinary Academic Research
(HSR-ICMAR) 2026
https://houghtonstreetreview.com/index.php/conference

BIOFILM FORMATION STAGES

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https://doi.org/10.5281/zenodo.21551123

Abstract

Biofilm formation is a dynamic and highly organized process that enables microorganisms to survive under unfavorable environmental conditions and resist antimicrobial treatment. Biofilms consist of microbial communities embedded within a self-produced extracellular polymeric substance (EPS) matrix that protects cells from antibiotics, disinfectants, and host immune responses. This study reviews the sequential stages of biofilm formation, including reversible attachment, irreversible adhesion, microcolony formation, maturation, and dispersion. The biological mechanisms underlying each stage, such as bacterial adhesion, EPS production, quorum sensing, and differential gene expression, are discussed together with their contribution to microbial persistence and antimicrobial resistance. The review also highlights the clinical significance of biofilm-associated infections, particularly those involving chronic wounds, implanted medical devices, urinary catheters, prosthetic joints, and cardiovascular implants. Recent advances in anti-biofilm strategies, including quorum sensing inhibitors, antimicrobial peptides, bacteriophage therapy, nanotechnology-based drug delivery systems, and enzymatic degradation of the biofilm matrix, are briefly summarized. A comprehensive understanding of biofilm development provides valuable insights into the prevention and management of persistent microbial infections and supports the development of more effective therapeutic approaches for combating biofilm-related diseases. (PMC OBJ)

Keywords: Biofilm formation; extracellular polymeric substances (EPS); bacterial adhesion; quorum sensing; antimicrobial resistance; microcolony; biofilm maturation; biofilm dispersion; chronic infection; medical devices.

Introduction

Biofilms are highly organized communities of microorganisms that attach to living or non-living surfaces and become embedded within a self-produced extracellular polymeric substance (EPS) matrix. This matrix, primarily composed of polysaccharides, proteins, lipids, and extracellular DNA, provides structural integrity and creates a protective microenvironment that enhances microbial survival under adverse conditions [1]. Biofilm formation is considered one of the most successful adaptive strategies employed by bacteria, fungi, and some archaea, enabling them to withstand environmental stress, nutrient limitation, antimicrobial agents, and host immune defenses [2].Unlike planktonic microorganisms, which exist as freely suspended cells, microorganisms within biofilms exhibit altered gene expression, metabolic activity, and intercellular communication through quorum sensing. These physiological changes contribute to increased tolerance to antibiotics and disinfectants, making biofilm-associated infections particularly difficult to eradicate [2]. The close spatial organization of microbial cells also facilitates horizontal gene transfer, including the dissemination of antimicrobial resistance genes, thereby posing a significant challenge to modern healthcare systems [3].

Biofilms are widely distributed in natural, industrial, and clinical environments. In medicine, they are responsible for numerous persistent and chronic infections, including catheter-associated urinary tract infections, prosthetic joint infections, infective endocarditis, chronic wound infections, ventilator-

associated pneumonia, dental plaque, and infections involving implanted medical devices [1,3]. According to current estimates, biofilms are implicated in approximately 65–80% of microbial infections, emphasizing their substantial impact on patient morbidity, healthcare costs, and treatment outcomes [2].

The development of a biofilm is a dynamic and highly regulated process involving multiple sequential stages, including initial reversible attachment, irreversible adhesion, microcolony formation, maturation, and dispersion. Each stage is controlled by intricate molecular mechanisms involving surface adhesins, extracellular polymeric substance production, quorum sensing pathways, and environmental signals [1]. A comprehensive understanding of these developmental stages is essential for identifying novel therapeutic targets, improving infection prevention strategies, and developing innovative anti-biofilm agents capable of overcoming conventional antimicrobial resistance [2,3].Therefore, the present study aims to review the sequential stages of biofilm formation, describe the biological mechanisms involved in each stage, and highlight their clinical significance in the prevention and management of biofilm-associated infections.

Main Part

1. Initial Reversible Attachment

Biofilm formation begins with the initial attachment of planktonic microorganisms to a surface. This stage is generally reversible because the interactions between microbial cells and the substrate are relatively weak. The attachment process is influenced by numerous physicochemical factors, including surface hydrophobicity, roughness, electrostatic forces, temperature, pH, nutrient availability, and fluid shear stress. Both biotic surfaces, such as epithelial tissues, and abiotic surfaces, including catheters, prosthetic implants, and medical devices, can serve as suitable substrates for microbial adhesion [4].

During this phase, microorganisms approach the surface through passive transport, Brownian motion, sedimentation, or active motility mediated by flagella. Initial contact is primarily governed by long-range attractive and repulsive forces described by the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory. Although these interactions are relatively weak, they allow microorganisms to remain in close proximity to the surface long enough to evaluate environmental conditions before establishing permanent adhesion [4].Bacterial appendages, including flagella, pili, and fimbriae, play an essential role in reversible attachment. Flagella facilitate motility and enable bacterial cells to overcome hydrodynamic barriers, while pili and fimbriae mediate transient interactions with host tissues and artificial materials. Gram-positive bacteria frequently rely on surface proteins known as microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), whereas Gram-negative bacteria often utilize pili and fimbrial adhesins to initiate colonization [5].

Environmental conditions strongly influence the success of initial attachment. Nutrient-rich environments promote bacterial metabolism and increase the likelihood of adhesion, whereas unfavorable environmental conditions may prevent stable attachment. Likewise, surface characteristics significantly affect microbial colonization. Rough surfaces provide greater surface area and protective niches, making bacterial attachment easier than on smooth materials. Hydrophobic surfaces often enhance adhesion of hydrophobic bacterial species, whereas hydrophilic coatings may reduce bacterial colonization [4].

Host-derived conditioning films also contribute to early adhesion. Within minutes after implantation of a medical device, plasma proteins such as fibrinogen, fibronectin, collagen, albumin, and laminin rapidly adsorb onto the material surface. These proteins create binding sites for bacterial adhesins, thereby facilitating microbial attachment. Consequently, bacterial adhesion frequently occurs not directly to the biomaterial itself but to the adsorbed host protein layer [5].At this stage, attached microorganisms remain metabolically similar to planktonic cells and can still be removed

through mechanical forces, immune responses, or appropriate cleaning procedures. Therefore, preventive strategies aimed at disrupting reversible attachment are considered one of the most effective methods for controlling biofilm-associated infections.

2. Irreversible Attachment

If favorable environmental conditions persist, reversible attachment gradually progresses into irreversible adhesion. During this transition, microorganisms begin producing extracellular polymeric substances (EPS), a highly hydrated matrix composed primarily of polysaccharides, proteins, extracellular DNA (eDNA), lipids, and other biomolecules. The EPS matrix anchors microbial cells firmly to the surface and represents the structural foundation of the developing biofilm [6].

Simultaneously, bacterial gene expression undergoes substantial changes. Numerous genes involved in adhesion, EPS biosynthesis, stress tolerance, nutrient acquisition, and biofilm development become activated. Cellular communication through quorum sensing also begins to coordinate the behavior of neighboring cells. Autoinducer molecules accumulate as bacterial density increases, allowing microorganisms to synchronize EPS production, regulate virulence factors, and initiate multicellular organization [6].Extracellular DNA serves multiple functions during irreversible attachment. Besides contributing to the structural stability of the matrix, eDNA enhances bacterial aggregation and facilitates horizontal gene transfer between neighboring cells. This process promotes the dissemination of antimicrobial resistance genes and virulence determinants, increasing the adaptive capacity of biofilm-associated microorganisms [5].

Once irreversible attachment has been established, bacterial cells become significantly more resistant to environmental stress than their planktonic counterparts. The developing EPS matrix limits the penetration of antimicrobial agents and shields microorganisms from host immune defenses. As a result, eliminating bacteria after irreversible adhesion becomes considerably more difficult than during the initial attachment phase [4–6].The irreversible attachment stage therefore represents a critical turning point in biofilm development. Successful interruption of EPS synthesis or inhibition of quorum sensing during this phase may prevent further biofilm maturation and substantially reduce the risk of chronic, device-associated, and recurrent infections.

3. Microcolony Formation

Following irreversible attachment, bacterial cells begin to proliferate and organize into small, densely packed clusters known as microcolonies. This stage marks the transition from individual surface-associated cells to a multicellular microbial community. Cell division, together with continuous production of extracellular polymeric substances (EPS), promotes the expansion of these aggregates and strengthens their attachment to the surface. The developing EPS matrix functions as a scaffold that maintains structural stability while facilitating interactions among neighboring cells [7].

Microcolony formation is accompanied by significant physiological changes. Bacterial cells alter their patterns of gene expression, enabling adaptation to the biofilm lifestyle. Quorum sensing becomes increasingly active as the concentration of signaling molecules rises with cell density. Through quorum sensing, microorganisms coordinate EPS production, regulate metabolic pathways, and synchronize the expression of virulence factors. This coordinated behavior enhances the survival of the microbial community under stressful environmental conditions [7].As microcolonies enlarge, gradients of oxygen, nutrients, and metabolic waste products begin to develop within the biofilm. Cells located near the surface generally have greater access to oxygen and nutrients, whereas deeper layers experience nutrient limitation and reduced metabolic activity. These physiological differences create heterogeneous bacterial populations that exhibit varying levels of antimicrobial susceptibility. Slow-growing or dormant cells in deeper regions often demonstrate increased tolerance to antibiotics, contributing to the persistence of chronic infections [8].

4. Biofilm Maturation

The maturation stage represents the most complex phase of biofilm development. During this process, the microbial community evolves into a highly organized three-dimensional structure composed of multiple layers of cells embedded within an extensive EPS matrix. Mature biofilms frequently exhibit mushroom-like or tower-shaped architectures separated by water channels that facilitate the transport of nutrients, oxygen, signaling molecules, and metabolic waste throughout the community [7].

The extracellular polymeric matrix constitutes the majority of the biofilm biomass and performs numerous protective functions. It acts as a physical barrier that restricts the penetration of antimicrobial agents, disinfectants, antibodies, and immune cells. In addition, the matrix protects microorganisms against dehydration, oxidative stress, mechanical disruption, and fluctuations in environmental conditions. These protective properties explain why mature biofilms are considerably more difficult to eradicate than planktonic bacterial populations [8].Within mature biofilms, microorganisms establish complex ecological interactions. Different bacterial species may cooperate through metabolic cross-feeding, while others compete for nutrients and space. Horizontal gene transfer occurs more efficiently because of the close proximity of cells and the presence of extracellular DNA. Consequently, antimicrobial resistance genes and virulence determinants can spread rapidly throughout the microbial community, further increasing treatment challenges [7].

The metabolic diversity observed within mature biofilms also contributes to treatment failure. Some bacterial cells remain metabolically active and proliferate rapidly, whereas others enter dormant or persister states characterized by extremely low metabolic activity. Because many antibiotics primarily target actively dividing cells, dormant populations often survive antimicrobial therapy and subsequently regenerate the biofilm after treatment has ceased [8].

5. Dispersion

Dispersion is the final stage of the biofilm life cycle and plays a crucial role in the dissemination of microorganisms to new environments. During this phase, individual cells or small clusters detach from the mature biofilm and return to the planktonic state. Dispersion may occur in response to nutrient depletion, accumulation of toxic metabolites, changes in oxygen availability, mechanical forces, or alterations in environmental conditions. In addition, microorganisms actively regulate dispersion through enzymatic degradation of the EPS matrix and quorum sensing-controlled signaling pathways [7].

Dispersed cells possess enhanced colonization potential and can rapidly attach to new surfaces, initiating another cycle of biofilm formation. In clinical settings, this process contributes to the spread of infection from contaminated medical devices to surrounding tissues or the bloodstream. Biofilm dispersion is therefore associated with recurrent infections, bacteremia, and the colonization of additional implanted biomaterials [8].Understanding the mechanisms responsible for biofilm dispersion has important therapeutic implications. Strategies that either inhibit bacterial attachment during the early stages or induce controlled biofilm dispersal in combination with effective antimicrobial therapy are being actively investigated as promising approaches for preventing and treating biofilm-associated infections.

Clinical Significance

Biofilm formation has become one of the major challenges in modern medicine because it significantly reduces the effectiveness of antimicrobial therapy and promotes persistent infections. Biofilms are commonly detected on urinary catheters, central venous catheters, orthopedic implants, prosthetic heart valves, contact lenses, and other indwelling medical devices. Their presence is associated with prolonged hospitalization, increased healthcare costs, repeated surgical interventions, and elevated morbidity [7].Advances in biofilm research have encouraged the development of innovative preventive and therapeutic strategies, including antimicrobial surface coatings, quorum sensing inhibitors, bacteriophage therapy, antimicrobial peptides, nanoparticles, and enzymes capable

of degrading the extracellular polymeric matrix. A comprehensive understanding of every stage of biofilm development is essential for designing more effective methods to prevent microbial colonization and improve the clinical management of biofilm-associated infections [8].

Conclusion

Biofilm formation is a complex, dynamic, and highly regulated biological process that enables microorganisms to survive under adverse environmental conditions and resist antimicrobial treatment. The sequential stages of biofilm development—including reversible attachment, irreversible adhesion, microcolony formation, maturation, and dispersion—are coordinated through intricate molecular mechanisms involving extracellular polymeric substance (EPS) production, quorum sensing, and differential gene expression.

The unique structural organization and physiological heterogeneity of biofilms significantly contribute to their increased tolerance to antibiotics, disinfectants, and host immune responses. Consequently, biofilm-associated microorganisms are responsible for many chronic, recurrent, and device-related infections that remain difficult to eradicate using conventional antimicrobial therapy.

A comprehensive understanding of the mechanisms underlying each stage of biofilm formation is essential for developing innovative preventive and therapeutic strategies. Emerging approaches, such as anti-adhesion surface modifications, quorum sensing inhibitors, bacteriophage therapy, enzymatic degradation of the EPS matrix, antimicrobial peptides, and nanotechnology-based drug delivery systems, offer promising opportunities for improving the prevention and treatment of biofilm-associated infections. Continued research into biofilm biology will contribute to more effective clinical management, reduced antimicrobial resistance, and improved patient outcomes.

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