ORIGINAL RESEARCH ARTICLE

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Biofilm Formation During Reprocessing of Reusable Medical Devices and Strategies for Its Elimination

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Abstract: Device-Associated Infections (DAIs) represent a significant global challenge to healthcare safety, and the formation of biofilms is widely recognized as one of their core pathogenic mechanisms. Throughout the entire process of cleaning, disinfection, and sterilization of medical devices (i.e., reprocessing), improper operation or procedural deficiencies may allow residual organic matter and microorganisms to adhere to device surfaces and rapidly develop into highly resistant biofilms. These biofilms act as persistent "hidden contamination sources," posing serious threats to patient safety. This paper systematically elaborates on the fundamental concepts and formation mechanisms of biofilms, as well as their dynamic evolution during each stage of medical device reprocessing (pre-treatment, cleaning, disinfection, sterilization, and drying/storage). It further analyzes key risk factors contributing to biofilm formation within current reprocessing workflows and comprehensively reviews recent advances and practical applications of physical, chemical, and emerging biofilm removal strategies. The aim of this study is to provide theoretical support and practical guidance for optimizing medical device reprocessing procedures, enhancing infection control, and safeguarding patient safety.

Keywords: medical devices; reprocessing; biofilm; infection control; cleaning and disinfection; removal strategies

Introduction

With the rapid advancement of modern medicine, invasive diagnostic and therapeutic procedures have become increasingly common, and sophisticated medical devices are widely utilized in clinical practice. However, after use, these devices are frequently contaminated with blood and other organic substances and may carry a substantial microbial load. If reprocessing is incomplete or inadequate, microorganisms can colonize device surfaces and subsequently form biofilms. The World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) have identified biofilms

as a primary cause of recurrent and difficult-to-eradicate device-associated infections. Biofilms are not merely simple aggregates of microorganisms; rather, they are structured microbial communities encased within an extracellular polymeric substance (EPS) matrix, characterized by complex three-dimensional architecture and distinct physiological functions. This structural organization confers enhanced environmental adaptability and markedly increased resistance to external stressors, including antibiotics, disinfectants, and host immune responses. Microorganisms embedded within biofilms may exhibit resistance levels 10–1000 times greater than those of their planktonic counterparts. Conventional reprocessing methods often fail to effectively eliminate biofilm-associated microorganisms, leading to potential "false-negative" detection results and an increased risk of cross-infection. Therefore, gaining a comprehensive understanding of the mechanisms underlying biofilm formation throughout the entire medical device reprocessing cycle, as well as developing efficient strategies for their removal and prevention, represents an urgent priority in the field of infection control. This paper presents a systematic discussion centered on this critical issue.

1. Fundamental Characteristics and Formation Mechanisms of Biofilms

1.1 Definition and Structure of Biofilms

A biofilm is a complex aggregate composed of microbial communities attached to inert or living surfaces and the extracellular polymeric substances (EPS) they secrete, forming a protected microenvironment. The typical architecture of a biofilm appears mushroom-shaped or columnar, with internal water channels that facilitate the transport of nutrients and the removal of metabolic waste. The EPS matrix is primarily composed of polysaccharides, proteins, nucleic acids (extracellular DNA, eDNA), and lipids. This matrix establishes both a physical and chemical barrier, serving as the material foundation for the high resistance characteristic of biofilms. By embedding microorganisms within this structured matrix, the biofilm provides mechanical stability, mediates adhesion to surfaces, and significantly enhances microbial tolerance to environmental stressors.

1.2 Kinetics of Biofilm Formation

Biofilm formation is a dynamic and multi-stage process that can generally be divided into the following key phases:

(1) Reversible attachment: Planktonic microorganisms temporarily adhere to the surface of medical devices—often pretreated yet still bearing trace organic residues—through weak physicochemical interactions such as van der Waals forces and electrostatic attraction.

(2) Irreversible attachment: Microorganisms anchor firmly to the surface via appendages such as flagella and pili and begin secreting small amounts of EPS. This secretion stabilizes surface adhesion and marks the transition from reversible to irreversible attachment.

(3) Microcolony formation and early development: The attached microorganisms proliferate and form small cellular clusters. During this stage, the Quorum Sensing (QS) system is activated, enabling microorganisms to coordinate collective behavior through signaling molecules [1].

(4) Maturation: Under QS regulation, microorganisms secrete large quantities of EPS, resulting in the development of a mature biofilm characterized by a complex three-dimensional architecture and integrated water channels. Different microbial species may coexist symbiotically, forming multispecies biofilms that further enhance structural stability and resistance.

(5) Dispersion: Portions of the mature biofilm detach, releasing planktonic microorganisms that colonize new surfaces and complete the biofilm life cycle.

Throughout the medical device reprocessing cycle, delays or operational errors at any stage may create a "window of opportunity" that facilitates one or more phases of biofilm development, thereby promoting its establishment and persistence.

2. Analysis of Biofilm Formation Risk Points Throughout the Reprocessing Workflow

2.1 From Post-Use to Pre-Treatment: A Critical Window for Biofilm Initiation

This stage represents the period of highest risk for biofilm formation. If medical devices are not promptly subjected to point-of-use pre-treatment—such as wiping with moist gauze or flushing with a dedicated moisturizing solution—residual blood, mucus, and other organic materials can rapidly dry and coagulate. These residues accumulate particularly within complex structures, including lumens, joints, and narrow crevices, forming a hardened "protective shell" that is

difficult to remove. This desiccated organic layer not only provides a nutrient-rich substrate for microbial growth but also significantly impedes the effective penetration of subsequent cleaning agents. Studies have shown that when contaminated devices are left at room temperature for more than one hour, common nosocomial pathogens, such as Staphylococcus aureus and Pseudomonas aeruginosa, may initiate irreversible attachment, thereby laying the foundation for rapid biofilm development. Therefore, immediate post-use intervention constitutes the first—and most critical—line of defense in interrupting the chain of biofilm formation during medical device reprocessing.

2.2 Cleaning Stage: A Contest Between Removal and Regeneration

As the core step of reprocessing, cleaning aims to physically eliminate all visible and invisible contaminants. However, this stage itself may harbor hidden risks for biofilm formation. For devices with highly complex structures—such as laparoscopes, bronchoscopes, and orthopedic powered instruments—manual brushing often fails to reach microscopic dead spaces. Automated cleaning systems, including ultrasonic cleaners and washer-disinfectors, can also yield suboptimal outcomes if operational parameters (e.g., time, temperature, detergent concentration) are improperly configured or if device loading is inappropriate. Even minimal organic residues can serve as a sufficient substrate for microbial adhesion and proliferation. Water quality used in cleaning is another critical factor. Minerals present in hard water may deposit on device surfaces as scale, creating a rough interface that facilitates microbial attachment. Moreover, if the water supply itself is contaminated with microorganisms, it may directly introduce the "seeds" of biofilm formation onto device surfaces [2]. Equally concerning is the interval following cleaning. If devices are not promptly transferred to the next reprocessing stage and remain in a moist environment for prolonged periods, both residual and newly introduced microorganisms can rapidly exploit the damp surface to initiate recolonization and biofilm reconstruction.

2.3 Disinfection and Sterilization Stage: The Challenge of Resistance

Once a biofilm reaches maturity, it poses a formidable resistance challenge to conventional disinfection and sterilization methods. High-level disinfection relies on chemical agents such as glutaraldehyde, ortho-phthalaldehyde (OPA), and peracetic acid. Although these agents exhibit potent microbicidal activity against planktonic microorganisms, their ability to penetrate the dense extracellular polymeric substance (EPS) matrix of mature biofilms is markedly limited. In many cases, disinfectants act only on the superficial layers of the biofilm, while microorganisms embedded in the deeper regions remain protected. Under favorable conditions, these surviving microorganisms can recover and proliferate, leading to persistent contamination. Even sterilization procedures—such as steam sterilization under high pressure or ethylene oxide (EtO) sterilization—which are theoretically capable of eliminating all forms of microbial life, are entirely dependent on a completely clean device surface. If a physical barrier composed of biofilm and residual organic matter is present, sterilizing agents cannot effectively contact and inactivate the target microorganisms, potentially resulting in catastrophic sterilization failure. For heat-sensitive medical devices that cannot tolerate high temperature and pressure, reprocessing relies predominantly on chemical methods. This reliance further increases the complexity and critical importance of accurately assessing and ensuring biofilm removal efficacy.

2.4 Drying and Storage Stage: The Risk of Secondary Contamination

The completion of reprocessing does not guarantee absolute safety. Thorough and complete drying is essential, as moisture is a fundamental requirement for microbial survival and proliferation. If the drying process is inadequate—leaving residual moisture on device surfaces or within lumens—or if packaging becomes damaged during storage, or if environmental humidity is excessively high, airborne microorganisms or contaminants originating from storage cabinets may recolonize device surfaces. This risk is particularly pronounced for devices kept in long-term storage. Although they may appear securely packaged and clean, new biofilms can silently develop within seemingly intact sterile barriers, ultimately serving as potential sources of future infections.

3. Existing and Emerging Strategies for Biofilm Removal and Prevention

3.1 Physical Removal Strategies

Physical approaches aim to disrupt biofilm structure

through mechanical forces and constitute the first line of defense in both prevention and removal. The core principle lies in optimizing manual cleaning procedures. This includes the use of dedicated brushes precisely matched to the dimensions of device lumens, application of correct brushing techniques (e.g., rotational and push–pull motions), and ensuring adequate cleaning duration. These measures are essential to guarantee effective treatment of every corner of complex medical devices. The enhanced application of automated cleaning technologies is equally critical. For instance, the cavitation effect generated by ultrasonic cleaning produces powerful shock waves capable of dislodging contaminants and early-stage biofilms from device surfaces and microscopic crevices. The combined use of multi-enzymatic detergents with ultrasonic systems may produce synergistic effects, further improving cleaning efficacy. Washer-disinfectors employ high-pressure water jets and turbulent flow to achieve comprehensive surface irrigation and mechanical flushing of instruments [3]. In addition, several advanced physical technologies have demonstrated significant potential in laboratory investigations. Techniques such as laser-induced breakdown spectroscopy (LIBS) and cold atmospheric plasma (CAP) have shown the capacity to effectively remove persistent biofilms without damaging device materials. Although large-scale clinical implementation remains in development, these technologies represent promising future directions in biofilm control.

3.2 Chemical Removal Strategies

Chemical approaches involve the application of specific agents to dissolve the EPS matrix, inactivate microorganisms, or interfere with microbial communication systems, thereby dismantling biofilm integrity. Specialized multi-enzymatic detergents can effectively degrade proteins, lipids, and carbohydrates that constitute the structural components of the biofilm matrix, eliminating its material foundation at the source. Alkaline cleaning agents are particularly effective against lipid-based contaminants, whereas acidic agents are suitable for removing inorganic salt deposits and scale. In recent years, a class of functional chemicals known as biofilm dispersants has attracted considerable attention. Examples include D-amino acids, nitric oxide (NO) donors, and certain targeted surfactants. Unlike conventional disinfectants, these agents do not primarily exert direct bactericidal effects. Instead, they disrupt microbial quorum sensing (QS) systems or selectively degrade key components of the EPS matrix (e.g., extracellular DNA or polysaccharides), thereby inducing structural disintegration of the biofilm. As a result, previously protected microorganisms become exposed, significantly enhancing the efficacy of subsequent disinfectants or antibiotics [4]. Simultaneously, the development of enhanced disinfectant formulations capable of penetrating the EPS matrix has become a research focus. Strategies include combining peracetic acid with specific surfactants, as well as employing advanced delivery technologies—such as microemulsions and nanocarriers—to facilitate targeted transport of active antimicrobial agents into the deeper layers of biofilms.

3.3 Integrated and Preventive Strategies

The most effective approach lies in the organic integration of physical and chemical methods throughout the entire reprocessing workflow, forming a comprehensive system centered on prevention. Mandatory implementation of immediate post-use treatment constitutes the cornerstone of this system. The application of specialized moisturizing solutions containing wetting agents and preservatives can effectively prevent organic residues from drying and inhibit early microbial adhesion, thereby fundamentally narrowing the “time window” for biofilm formation. Strict time management across the entire reprocessing cycle is equally indispensable. Clear regulations should define the maximum allowable interval from device use to the completion of sterilization, preventing prolonged stagnation at any intermediate stage.

From a source-control perspective, innovation in medical device materials represents a fundamental pathway. The development of devices coated with anti-biofilm surfaces—such as hydrophilic polymers (e.g., polyethylene glycol), antimicrobial peptides, or nano-silver particles—can render device surfaces less prone to microbial adhesion or confer intrinsic antimicrobial properties. Finally, establishing a comprehensive process monitoring and validation system serves as the “eyes” of the entire framework. Rapid methods such as adenosine triphosphate (ATP) bioluminescence assays and residual protein detection should be employed for real-time monitoring of cleaning efficacy. In addition, biological indicators (BI) and chemical indicators (CI)

should be regularly utilized to verify the effectiveness of disinfection and sterilization processes, ensuring that every stage remains under controlled conditions.

Conclusion

Biofilm formation during medical device reprocessing constitutes a complex systemic challenge involving microbiology, materials science, engineering, and management disciplines. Owing to their unique structural and physiological characteristics, biofilms present significant resistance to conventional reprocessing methods and represent a critical underlying cause of persistent Device-Associated Infections (DAIs). By analyzing the mechanisms of biofilm formation, this paper has identified key risk points throughout the reprocessing workflow and systematically reviewed physical, chemical, and integrated strategies for biofilm removal and prevention.

Future research should focus on the following directions: (1) Developing more sensitive and rapid biofilm detection and monitoring technologies to enable early warning in clinical practice; (2) Further elucidating the interaction mechanisms within multispecies biofilms and their responses to reprocessing interventions; (3) Accelerating the clinical translation of novel anti-biofilm materials and efficient, environmentally sustainable removal agents.

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