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Biofilms on Voice Prosthesis – Challenges and Therapeutic Insights

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ABSTRACT

After a total laryngectomy, voice rehabilitation is essential for a patient to successfully reintegrate into society. The implantation of a voice prosthesis (VP) is the gold standard to achieve this goal. Thus, the primary disadvantage of using VP is the fluid blockage and degradation caused by biofilm colonization, which requires frequent replacements, associated with a poor quality of life for the patient. Many scientists have centered their research on coming up with novel and efficient ways to combat polymicrobial biofilms, both in terms of preventing microbial adhesion and rupturing established biofilms in order to overcome this limitation. This paper aims to present the current state of the art regarding biofilm formation on VPs and composition of VPs, and to review the current anti-biofilm strategies that have proven to be successful, as well as pointing possible novel perspectives of improvement.

Keywords: voice prosthesis, biofilm, inhibition strategies.

INTRODUCTION

The main aspect that impacts the quality of life on laryngectomized patients is the loss of voice function. The ability to regain this function by vocal rehabilitation is now the gold standard for patients who underwent total laryngectomy and its aim is to improve their quality of life, overcoming the repercussions of disability in social, physical, psychological and even vocational area (1, 2). There are three main options for vocal rehabilitation, including esophageal speech, use of electronic devices (electrolarynx) and implantation of voice prosthesis (VP) through tracheoesophageal puncture (TEP). While each approach has advantages and drawbacks, the use of VP is by far the preferred choice for voice rehabilitation due to its superior resemblance to physiological voice (3), with an encouraging success rate of about 87% (4).

Various types of VP proved their reliability for vocal rehabilitation, with comparable device life span and quality of obtained voice (5). Besides advantages, there are also considerable complications associated to the implanted VPs such as biofilm settlement on the prosthesis surface, that is composed mainly of silicone rubber, leakage, prosthesis migration, granulation tissue development or inflammation at the puncture site. Biofilm coloni-

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zation represents the main limitation for VP use due to its negative effects. Thus, the rapid deterioration of the rubber and the increased resistance to the airflow caused by biofilm development leads to improper function, thus requiring more frequent replacements and, furthermore, negatively impacting the quality of life. In this regard, the aim of the present paper is to outline the currently used antimicrobial strategies that showed efficacity on preventing or limiting biofilm formation on VPs.

Steps in biofilm formation

Bacterial biofilms have emerged as a threat to the global health services. They are a potential source of infection, which is difficult to address because of resistance to antibiotic use and ability to intrude the defense immune system of the host. The common sites for biofilm development include the surface of medical instruments, various accessory medical devices (pacemakers, intravascular respectively urinary catheters, endotracheal tubes, voice prosthesis) and body tissues, but their adaptability mechanisms provides all elements for their omnipresence in natural environments (6).

The general structure of biofilm consists of self-generated matrix (more than 90% of the biofilm biomass) made up mainly of extracellular DNA, polysaccharides and other elements (biopolymers), whereas microorganisms (bacteria, fungi or both) sum up to less than 10% of the dry mass.

Biofilm development is a process organized in five stages: 1) attachment of bacteria to a surface; 2) irreversible adhesion; 3) generation and secretion of extracellular polymeric substance (EPS) by the microbial cells; 4) maturation; and 5) biofilm diffusion (7).

Cell migration and adherence to a surface are the initial events in the first stage, which can be reversed. If the surrounding conditions are beneficial, adherent cells cause the biofilm to develop on the surface and produce their shield using small amounts of exopolymeric material. In the second stage, the adherent cells that are now irreversibly bonded to the surface release the extracellular exopolymeric substance, which promotes cell aggregation and matrix development. The third stage of development is the maturation of the biofilm, with the multi-layer display signifying the architectural progress brought on by colony growth and water channel construction. In the fourth stage, the biofilm reaches its highest cell density and becomes a three-dimensional community. The process is completed during the fifth stage, when the mature biofilm enhances the production and distribution of microcolonies of cells from the central community, which travel to adjacent surfaces, expanding the colonization and subsequently the infection to other areas (7, 8).

The ability of microorganisms growing in biofilms to adapt to different environmental factors is unique because it depends on quorum sensing (QS), a density-dependent mechanism that entails the exchange of chemical substances and signaling coupled to an electrical impulse (9). Quorum sensing regulates the expression of genes that affect virulence, competition, pathogenicity, and resistance by detecting cell density through chemical signals made up of minute amounts of chemical compounds (commonly referred to as autoinducers) (10).

Biofilm composition on VPs

Bacteria and fungi are the most frequently identified types of organisms in VPs biofilms, and some of these microorganisms are capable of penetrating the silicone rubber directly. An interesting question is whether the order of colonization or microbial composition is important; according to most research in the field, it seems that bacteria adhesion to VP surface is essential to provide the optimal environment for consecutive yeast colonization (11, 12). Additionally, the synergistic relationship between *S. aureus*, otherwise a component of the oral and perioral microbiota, and *C. albicans* species has been documented in a previous study, which revealed that *S. aureus* was found on 64.2% of dysfunctional VPs and was recurrently associated with only one or various Candida species (13).

Regarding multi-layered distribution, on the majority of prostheses examined by Buijssen *et al* (14), yeast colonies could be seen growing inside the silicone rubber and bacteria were primarily found close to the outermost surface of the biofilm. Furthermore, it has been acknowledged that oral anaerobic microorganisms tended to cluster in the valve of the VP, supposably because of the biofilm stability in that area in comparison with other VP parts. Noteworthy, besides of being part of the commensal oral flora, microcolonies of *Fusobacterium nucleatum* appear to have a contribution to biofilm formation on VPs, being identi-

fied on devices that surpass an average of 170 days of use (15).

Considering that fungal species in general, and Candida albicans in particular, were long-term recognized as being a leading component in biofilm formation on VPs, the majority of *in vitro* studies focused on assessing the fungal biofilm model on growth media. More recently, advances in biofilm research have revealed the remarkable networking of mixed bacterial and fungal structure as well as complex interactions based on QS that strengthen proliferation, diffusion and overall survival ability (16).

C. albicans is recognized to be the most common fungus pathogen causing serious hospital acquired infections. It has also been observed to create polymicrobial biofilms in cohabitation with bacteria, particularly *Streptococcus* spp., *Pseudomonas aeruginosa*, *S. aureus* and *S. epidermidis* (17). Other commonly identified species of fungi on used VPs are represented by *Candida krusei*, *Candida glabrata*, *Candida tropicalis* and *Saccharomyces cerevisiae* (14, 18).

In another study, the relationship between substandard VP lifespan and mixed polyspecies fungal and bacterial colonization was also emphasized. Interestingly, the similarity of pathogens commonly found in the oral flora and VP microflora pointed out that oral decontamination regimens might prolong the VP use to 1.4-fold, being more favorable than non-specific antifungal therapy (19).

These multi-species communities make the struggle against biofilms increasingly challenging because they limit the efficacy of currently developed species-specific biofilm targeting strategies in addition to requiring the use of effective antibiotics against all pathogenic microorganisms which are present in the microbial community (20).

Anti-biofilm strategies

In order to elongate the lifespan of VPs, it is mandatory to identify and use agents or mechanisms that inhibit or partially reduce the development of biofilm formation. Conventional methods that only target antibacterial activity or EPS breakdown may not be effective in the complex (physicochemical) biofilm setting. In order to eliminate the pathogenic niche with the least amount of harm to neighboring tissues, potential therapeutic techniques must simultaneously target the biofilm matrix components and embedded microorganisms (21).

Several different strategies have been proposed to prevent or reduce the production of biofilms. Among them, the most commonly tested and used ones are the modification of silicone rubber surfaces, the use of nanoparticles or biosurfactants, or the prophylactic treatment with various antimicrobial agents. While the knowledge gap must be filled by better understanding the behavior and the adaptation mechanisms of biofilms, it leaves nevertheless ground for developing promising and innovative combative techniques.

The surfaces of these materials must be biocompatible, restrict the growth of biofilm, and support regeneration. To help the implants work and ensure a long lifespan, a variety of surface modification techniques and functionalization treatments can be used.

A meta-analysis of 33 comparative studies on biofilm inhibition using passive, active and combined surface modification demonstrated noteworthy differentiation in favor of fungal biofilm inhibition opposed to bacteria. The application of combined active and passive covering adaptation techniques was proven to have a superior inhibition potentiality (22).

There is no known way to prevent native microbiological species from the upper airways from colonizing polymer surfaces, and the only method that has proven to be successful so far is manipulating the chemical-physical characteristics of the polymer used in the device, as discussed previously. A recent study demonstrates that the fluoroplastic (teflon-like) Provox ActiValve exhibits superior clinical resistance to biofilm and a longer lifespan, being less susceptible to *in vitro* colonization by *Candida* species. The understanding of the bacterial and mycotic flora in biofilm colonization appears to be greatly improved by sonication. Designing a cleaning tool that can reach and scrub the esophageal flange of the prosthesis while yet protecting the valve mechanism could be a simple and useful solution to this issue (23).

Teflon and silver oxide have both been shown to minimize the formation of long-lasting biofilms when used as valve flap materials. The used model enables quick screening for innovative biofilm-inhibitive materials and robust coatings intended for medical equipment with increased biofilm resistance (24).

For the purpose of creating effective delivery systems for both natural and synthetic medicines, a variety of nanoparticles (NPs) can be used (25, 26).

These NPs have the potential to disrupt biofilms and internalize pathogenic microorganisms while also increasing the activity of the carried load, and assuring a continuous release of drugs at the target-site (27). Antimicrobial-loaded NPs can be used as efficient therapeutics delivered using a variety of routes, but they can also be further incorporated into biomaterials to alter surface nanotopography or coat biomedical devices with the goal of enhancing or inducing anti-infective properties (28, 29).

At present there are a number of nanosystem strategies, but the major one is the use of magnetic nanoparticles (MNPs), which can affect microbial cell sensitivity, thus preventing biofilm formation. By boosting peptide transport into cells and dislocating the fungal membrane, MNPs also strengthen the fungicidal action. This strategy effectively responds when paired with antibiotics or other natural antimicrobials, producing various outcomes for the selected approach. Although antimicrobial peptides are a costly alternative that potentially lessen drug resistance, antibiotics can still be used in this situation. Lower doses of antibiotics can be used, which slows the development of antimicrobial resistance (30).

The functionalization of cellulose, a natural biopolymer, converted to bacterial nanocellulose (BNC) with poly([2-(methacryloyloxy)ethyl]-trimethylammonium chloride) (PMETAC) exhibited inactivating effects on polymorphic *Candida albicans* fungal species. In vitro cytotoxicity evaluation revealed that the obtained nanocomposite had no toxicity against human keratinocytes (31).

The co-immobilization of cellobiose dehydrogenase (CDH) and deoxyribonuclease I (DNase) on positively charged chitosan nanoparticles (CSNPs) ensured the formation of a bifunctional nanoparticle (CSNP-DNase-CDH) that disrupts both microorganisms and biofilm formation. The assessment of in vitro antibiofilm proprieties of CSNPs against monomicrobial and polymicrobial biofilms of *Candida albicans* and *Staphylococcus aureus* enhanced their ability to penetrate the biofilm matrix and their potential to inhibit the proliferation of fixed microorganisms, as well as disorganizing the pre-existing biofilm (32).

Another favorable result was represented by the modification of polydimethylsiloxane membranes surface (PDMS) that preserved the material biocompatibility while lowering their hydrophobic proprieties and bacterial adhesion and respectively growth on its surface (33). Likewise, the screening of several meth-acrylate polymers served to identify specific polymer functional groups that were associated with fragile attachment and up to 100% reduction in *Candida albicans* biofilm compared to commercial materials (34).

In the direction of restraining the adherence of oropharyngeal microorganisms, the use of a colloidal palladium/tin solution for coating the VP silicone rubber surface demonstrated promising results for increasing the period of indwelled VP use (35).

Addressing the prevention of *C. albicans* attachment on acrylic resins, several chemical or physical surface alterations were convenient in particular modification of surface charge, expanding surface wettability or reducing surface energy, all of these methods providing lower *C. albicans* adhesion.

An attractive proof of concept states that covering VPs with an ongoing release layer containing the antifungal medication CTZ (clotrimazole) reduces the growth of biofilm on the VPs and offers long-term defense against fungus infections for the environment. This method ought to greatly increase the VP lifespan and enhance patient clinical results (36).

Water-soluble synthetic peptidomimetic polyurethanes disrupt biofilms of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*, which are resistant to conventional antibiotics, namely ciprofloxacin or polymyxin B. Despite the poor antimicrobial activity against planktonic bacteria, they prevent surface attachment and stimulate bacterial motility, inhibiting biofilm formation at subinhibitory concentrations and showing no toxicity to human cells. These polyurethanes have potential as therapeutics targeting biofilms and modulating surface interactions for chronic infections and antibiofilm agents (37).

Sodium selenite is another compound that has proven its efficiency in removing and inhibiting mature mixed-bacteria biofilms on VPs, also reducing biofilm formation potential and metabolic activity. The results of the study led by Zhang *et al* (38) enhanced the anti-biofilm proprieties of sodium selenite application *in vitro*, providing the starting point for novel strategies to prevent and eliminate *in vivo* biofilm formation on VPs and having clinical prospective.

An ingenious approach is represented by the incorporation of synthetic drugs (levofloxacin, ge-

ntamicin, ampicillin) or even natural antimicrobials into nanosized polymeric structures. Phytotherapy offers a generous range of natural substances with proven anti-biofilm proprieties (39) such as *Cymbopogon citratus* oil – with anti *C. tropicalis* biofilm effects (40), Plectranthus amboinicus essential oil – effective on vancomycin resistant *S. aureus* (41), oregano oil (42), red propolis extract (43), or *Cinnamomum zeylanicum* essential oil (44) .

Recently, it has been suggested to cover implantable materials with biosurfactants (BSs), a new category of highly biocompatible antiadhesive and antibacterial chemicals, in order to enhance their anti-biofilm capabilities. Through quantitative and qualitative *in vitro* tests, the anti-biofilm activity of lipopeptide AC7BS, rhamnolipid R89BS, and sophorolipid SL18 was assessed against clinically significant fungal/bacterial dual-species biofilms (*Candida albicans*, *Staphylococcus aureus*, *Staphylococcus epidermidis*). Dense biofilms could be created by *C. albicans-S. aureus* and *C. albicans-S. epidermidis* cultures on the surface of polystyrene plates and on silicone discs made of medical-grade silicone. Up to 72 hours on, all evaluated BSs showed effective inhibitory efficacy against the development of dual-species biofilms in terms of total biomass, cell metabolic activity, microstructural architecture, and cell viability (45).

Novel alternatives for specifically addressing anti-*S. aureus* biofilm are represented by bacteriocins, treatment with bacteriophages, the use of antibodies, nanotechnology or photoinactivation. The principle behind photodynamic inactivation (PDI) is to employ oxygen, a photosensitizer, and visible light to trigger a phototoxic reaction that destroys bacteria. As already established, the generation of staphylococcal biofilms depends on cell-to-cell communication via quorum-sensing (QS), which makes this system a desirable target for antibiofilm drugs (46).

Future directions

Although many of the previous investigations failed to advance to in vivo trials and even fewer to clinical application, the great majority of studies were carried out in vitro utilizing models or treatment regimens that did not progress beyond the preclinical stage. As a result, careful additional research is necessary before they are made available in clinical and commercial settings. Development of antimicrobial drugs for a specific microbial species is very difficult due to the complexity of host microbiota, where commensals co-exist with potential infections. Another difficulty arises from the presence of biological fluids that alter surface chemistry.

Further investigations should focus on addressing complex and mixed biofilms, a difficult and understudied subset of microbial diseases. Prophylaxis of biofilm development is still a challenge for many medical fields and calls for additional study in order to create more effective techniques to prevent its settlement. The development of improved anti-biofilm agents, such as various coating nanostructures or targeted delivery antimicrobial agents, that would extend the exploitation duration of VPs should be the primary emphasis of novel polymeric materials employed in their manufacturing. ❑

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