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Chapter 4

Biophysical Effects on Chronic Rhinosinusitis Bacterial Biofilms

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1. Introduction

Chronic rhinosinusitis (CRS) is a common debilitating condition. In the United States alone according to recent data from the National Health Interview Survey CRS affects approximately 31 million people [1] resulting in an estimated annual cost of $6 billion. Bacterial biofilms have been implicated in the pathogenesis of CRS [2-4].

This literature review focuses on the effect of physical excitation by ultrasound and/or electric fields on bacterial biofilms. The associated research is based on the hypothesis that external forces applied by ultrasound and electric fields can alter the attachment forces of biofilms and possibly help patients with chronic infective diseases such as chronic rhinosinusitis.

Firstly, the role of bacterial biofilms in CRS is discussed identifying the major bacterial species involved in CRS (section 2). Secondly, the importance of the cyclic process of biofilm formation is reviewed focusing on the nature of the attachment and detachment process (section 3). Thirdly, treatments are categorized into medication and physical methods (section 4), with a major emphasis on ultrasound and the bioelectric effect. Finally, future directions on the use of ultrasound and electric fields, and their potential effects on biofilms are discussed (section 5).

2. Chronic rhinosinusitis

The respiratory system is divided into the upper and the lower airways. In the upper airways, the air is filtered, warmed and moistened by the nasal cavity, which is surrounded by
a ring of air-filled cavities called the paranasal sinuses. These consist of the maxillary, sphenoid, frontal and ethmoid sinuses (Fig. 1). The proposed functions of the sinuses include moisturizing and humidifying ambient air, acting as resonators for speech, lightening the weight of the skull bones, providing protection to the brain from trauma, and producing nitric oxide.

Rhinosinusitis is defined as a group of disorders characterized by inflammation of the nose and paranasal sinuses. Based on the duration of inflammation, rhinosinusitis is classified as acute (< 4 weeks), subacute (4-12 weeks) or chronic (>12 weeks). CRS symptoms have been classified as major, which includes facial pain or pressure, nasal obstruction or blockage, discolored postnasal drainage, hyposmia (a reduced ability to smell) and purulence in nasal cavity, or minor, which includes headache, fever, dental pain and ear pain [5].

While the pathology of CRS remains unclear and is described as multifactorial, bacterial biofilms have been implicated as a major contributing factor [2-4]. Bacteria are now recognized as existing in two forms: free floating (planktonic) or in sophisticated communities called biofilms. They have been defined as “a structured community of bacterial cells enclosed in a self-produced polymeric matrix adherent to an inert or living surface” [7].

2.1. Biofilms

Biofilms are composed of two major components: microorganism cells that account for less than 10% and a matrix of extracellular polymer substances (EPS) that account for more than 90% of biofilms [8-10]. The characteristics of the cells in biofilms differ from their planktonic counterparts [11]. EPS is considered the primary matrix material of biofilms and is comprised of 50-90% organic carbon [10]. The biofilm matrix is composed of secreted polymers (polysaccharides), lipids, proteins, DNA and RNA [12]. Normally biofilms protect their inhabitants against environmental and biological threats [13].
Bacteria in a biofilm communicate chemically by using molecular chemical signals, a phenomenon called quorum sensing. It plays a major role in structuring complex communities and regulates a variety of physiological functions in both Gram-negative and Gram-positive bacteria [14]. Physical signals may also be involved in bacterial communication [15], but this communication is not well understood.

The knowledge relating to the structure, function and mechanisms of quorum sensing is limited. It has been proposed that quorum sensing generates and responds to physical signals including sound waves, electric current and electromagnetic radiation [15]. Production of sound waves has been observed with Bacillus subtilis [16], however, measurable data have not been reported in the literature. Intensity limitations and time scale requirements are challenges in acquiring physical quantitative data from biofilms.

Generally, bacteria are described as negatively charged under most physiological conditions [17]. However, the net charge varies from species to species and is most likely influenced by culture conditions [18], age of the culture [19], ionic strength [20] and pH [21]. During the formation of biofilms, the net negative charge of the bacteria is reduced in order to modify their cellular surface charge [22]. This phenomenon has been observed with Staphylococcus aureus [23, 24]. Similarly, the measurement of surface charge of Staphylococcus epidermidis shows a variation in the net charge suggesting that different regions of the cell on the surface have different surface charges although the overall net charge may be negative [25]. The net charge has been decreased by increasing pH [21].

In addition to the biological and physical attributes of bacterial biofilms, they have a resistance to antibiotics 10-1000 times higher than planktonic bacteria of the same species [26]. A simple description of the biofilm resistance is the formation of a physical barrier to prevent antibiotics from penetrating biofilms [27]. Specifically, it has been reported that mechanisms include restricted penetration, enzymatic destruction of antimicrobial, gene transfer, quorum sensing, altered growth rate (persistor cells), stress response to hostile environmental conditions and over-expression of genes [28]. Multiple mechanisms may work together to increase bacterial biofilm resistance to antibiotics.

Several studies have investigated the relationship between biofilms and CRS [2, 3, 29, 30]. The most common bacterial biofilms in CRS are S. aureus, Pseudomonas aeruginosa, coagulase-negative staphylococci, Streptococcus pneumoniae, Moraxella catarrhalis and Haemophilus influenzae. Using scanning electron microscopy, Cryer et al. obtained the first evidence of biofilms in CRS showing the presence of P. aeruginosa biofilm in specimens taken from maxillary and ethmoid sinus mucosa [31]. Healy et al. investigated the presence of biofilms in CRS by using microscopic fluorescent in situ hybridization and showed that H. influenzae was most frequently present (80%) among the species identified, which included S. aureus, P. aeruginosa, S. pneumoniae and fungi [32]. However, confocal scanning microscopy and transmission electron microscopy studies have shown that S. aureus and P. aeruginosa respectively are the most common biofilm bacteria in CRS [33, 34]. Despite the uncertainty with respect to the dominant bacterial species of biofilms in CRS, they have been reported as the major cause of CRS. In addition, biofilms are found attached to sinus surfaces and/or aggregated with a surrounding mucus layer or fluid [35-37]. However, regarding the aggregation,
it is not well known whether they detach from surfaces, or whether aggregated biofilms form in the fluids themselves [38].

3. Biofilm formation

Before biofilms form, microorganisms move or are moved to a surface. Planktonic cells conventionally are thought to initiate contact with surfaces. However, this phenomenon and the mechanism behind it are not well understood. It is hypothesized to include random contact caused by several factors. These include Brownian motion, sedimentation owing to differences in specific gravity between the bacteria and the bulk liquid, convective mass transport in which cells are physically transported towards the surface, and active transport owing to the activity of the filamentous cell appendages such as flagella, in which chemotaxis may or may not be included [39].

The formation of a biofilm is divided into attachment, growth and detachment. Although the growth is a part of the cyclical process of biofilm formation, the focus will be on the physical mechanisms influencing the attachment and detachment of biofilms to surfaces.

3.1. Attachment

Several experimental and theoretical approaches have been used to investigate the attachment process. The process of attachment is generally divided into two stages, reversible and irreversible, or similarly named as docking and locking [13]. Numbers of environmental, physical, chemical and biological variables initiate the attachment, including extracellular DNA (eDNA), temperature, electrostatic charges and acid base interaction forces. The role of eDNA in the initial cell attachment is not fully understood, despite its role in the growth and development of *P. aeruginosa* biofilm [40, 41]. However, eDNA enhances the adhesion of Gram-positive bacteria [42].

Temperature effects appear to be different depending on the microorganisms. *P. aeruginosa* has been observed to adhere better to hydrophilic contact lenses at 37 °C than at 26 °C [43]. On the other hand, *S. pneumoniae* adhere better at 24° C than at 10 °C [44]. The temperature may cause an increase in nutrient intake by increasing the activity of enzymes in the production of EPS [45]. It has been observed that the temperature has an impact on the presence of bacterial surface appendages and a decrease in temperature may initiate adhesion [46]. The temperature may also change the environmental conditions surrounding the bacteria by changing the ionic strength of the polysaccharides [47].

A number of physical and chemical forces, including van der Waals, electrostatic charges and acid base interactions, initiate the attachment during the reversible stage [48]. Generally, van der Waals forces are described as an attractive force, while electrostatic forces are a repulsive force [49]. If the repulsive forces are greater than the attractive forces, small shear forces can detach bacteria from surfaces, unless a conditioning film on the substrate is formed by the presence of EPS [49, 50].
The transition from weak interactions (forces) of the cell with the substratum to a permanent bonding is mediated by the presence of EPS [51], which increases the strength of attachment [13]. In this transition, a process of attachment transfers from the reversible to the irreversible stage. Several forces, including covalent and hydrogen bonding as well as hydrophobic interactions, are generated [52]. In addition, electron transfer has been suggested as an extra step involved, by either donating electrons to, or accepting electrons from the substratum [53]. This might explain why the production of EPS is suggested to be mediated by electrostatic charges [54]. Electrostatic interaction forces contribute to the cross-linkers of the biofilm matrix [55]. Aggregated interaction (forces) between positive and negative charges are shown between exo-polysaccharide alginate of *P. aeruginosa* and extracellular enzymes [56]. Electrostatic interaction and hydrogen bonding forces are suggested to be the dominant forces in biofilm formation [57].

Bacterial adhesion forces change depending on the distance between the cells and the substratum. Force-distance relations are proposed to consist of three steps: van der Waals forces at a distance greater than 50 nm, van der Waals and electrostatic interaction between 10 and 20 nm and van der Waals, electrostatic interaction and other interactions at a distance less than 1.5 nm [58]. However, these physical forces have been further classified as long and short range interaction forces [59]. Long range occurs at a distance greater than 50 nm while short range occurs at less than 5 nm. Long and short range physical forces are related forces and highly dependent on the distance between the biofilm and a surface.

Adhesion forces have recently been measured using an atomic force microscope and optical tweezers [60-62]. However, a clear quantitative measurement of the bacterial forces has not yet been defined. Although all microscopic techniques including scanning electron microscopy, optical microscopy, and confocal laser microscopy do not quantitatively measure the adhesion forces, they qualitatively do observe the morphology of biofilms. Hence, a new technique is needed for accurately quantifying bacterial forces.

Although most of the above techniques were based on the experimental approaches, the theoretical analysis of bacterial adhesion is determined by three approaches: the Derjaguin-Landau-Verwey-Overbeek (DLVO) model, the thermodynamic approach and the extended DLVO theory. The DLVO model calculates the net energy of interaction between a cell and a surface [50]:

\[
\text{net energy of interaction} = \text{van der Waals energy} + \text{electrostatic energy}
\] (1)

However, this approach does not consider molecular and acid-base interactions. The thermodynamic approach calculates the surface free energies, assuming that the process is reversible only while it is described as distance dependent in the DLVO model. In addition, the thermodynamic system is assumed to be a closed system without any external input energy. Furthermore, electrostatic interactions are not taken into account in the surface free energies [63]. Neither the DLVO model nor the thermodynamic approach explain clearly the bacterial adhesion. Extended DLVO theory has been proposed recently by calculating the total adhesion energy in which the acid base interactions are included and given by:
\[ \Delta G^{\text{adh}} = \Delta G^{\text{vdW}} + \Delta G^{\text{dl}} + \Delta G^{\text{AB}} \] (2)

Where \( \Delta G^{\text{adh}} \): Total adhesion energy
\( \Delta G^{\text{vdW}} \): van der Waals interaction\( \Delta G^{\text{dl}} \): Electric double layer interaction\( \Delta G^{\text{AB}} \): Acid-base interactions

Although extended DLVO seems to be a promising theoretical approach, further investigation is needed to explain the exact mechanism of bacterial adhesion.

3.2. Detachment

The detachment of biofilms is influenced by several physical, chemical and biological conditions. This may lead to changes in the biofilm cells. Physical conditions play a major role in the bulk liquid surrounding biofilms and physical forces have been reported to change the structure of biofilms [64]. In addition, high shear forces have been shown to change the strength and weakness of biofilms [65]. Increasing the velocity and particle concentration of the liquid normally increases the detachment rate [66]. Shear forces influence biofilm thicknesses [51]; however variations in these forces depend on the biofilm formation [67, 68]. It has been observed that under steady shear forces that biofilms roll along surfaces [69].

A direct electric current was reported to enhance the detachment of *S. epidermidis* biofilm from surgical stainless steel [70]. However, a variation in the detachment rate was observed when the direction of the current was changed between electrodes at the same magnitude [71]. Direct current appears to cause a disruption of the electrical bi-layer on the substrate [72].

In addition to the above physical factors, the detachment of a biofilm can be attributed to changes in the chemical properties of the biofilm or surrounding media. pH has been shown to influence the structure of biofilms in a form of expansion and contraction [73]. The effect of nutrients on biofilm detachment is not well understood. Limitation of nutrients at the biofilm-liquid interface increases the detachment rate [74]; however, another study has shown that the increased availability of nutrients also increases the detachment rate [75].

Apart from the above physical and chemical changes, there are biological factors influencing the detachment process. These include polysaccharide, enzymes, genes and quorum sensing. While these factors have shown a marginal effect, surface-protein-releasing-enzyme produced by *Streptococcus mutans* has caused the release of biofilms from tooth surfaces at rates 20% higher than control samples [76].

Physical and biological parameters appear to be more significant than chemical ones despite the fact that the latter have an important role in the detachment process. The significance of the physical detachment is attributed to attachment forces; however, quorum sensing is biologically significant in structuring the biofilm community. A question worth raising is would a change in the physical parameters, such as frequency, break down and disrupt biofilm attachment to surfaces? Would these changes block quorum sensing in biofilms?
4. Methods of disrupting biofilms

Available methods of treatment for CRS bacterial biofilms are classified into two categories; conventional medication (biochemical) and biophysical.

4.1. Medication

Medication is the current recommended treatment method for CRS. However, they do not seem to improve long-term outcomes [77]. In addition, there are side effects [78] and there is a possibility of changing pathogens and resistance patterns that result in the persistence of CRS [79]. A problem of CRS antibiotic treatment is that these treatments are not able to eradicate bacteria in a biofilm state.

Alternative natural substances similar to antibiotics have been proposed such as honey and garlic. Manuka honey has been reported to be an effective treatment for CRS without damaging respiratory epithelium [80]. However, it does not block biofilm quorum sensing; in contrast, garlic has been shown to be effective in blocking quorum sensing [81]. Both conventional antibiotics and alternative natural substances are often ineffective against biofilms [82].

Vitamin D recently has been proposed as a treatment for CRS and to decrease the viability of *P. aeruginosa* biofilms [83]. However, the mechanism of action is unclear. As another treatment, baby shampoo as simple chemotherapy has been proposed for treating CRS [84]. However, side effects were observed in more than 10% of the patients who could not continue in the study.

4.2. Biophysical approach

Physical methods are classified into two categories: ultrasound and bioelectric effect.

4.2.1. Ultrasound

Ultrasound is a cyclic sound pressure utilizing frequencies higher than 20 kHz. It has been employed in a variety of medical applications ranging from diagnostic imaging to physical therapy [85]. Variables in ultrasound therapy include the type of ultrasound (continuous or pulsed), frequency, intensity, duty cycle, individual treatment duration and overall treatment length. Recently, it has been suggested that ultrasound could be a promising alternative method for treating biofilms [86].

*In vitro* [87] and *in vivo* research [88] has shown that ultrasound has a role in disrupting bacterial biofilms. It disrupts *Escherichia coli* [89], *S. epidermidis* [88], *P. aeruginosa* [90], coagulase-negative Staphylococci [87] and *S. aureus* [87] bacterial biofilms. Bacterial viability is further reduced when ultrasound is combined with antibiotics [91, 92]. The susceptibility of biofilms from different bacterial species to ultrasound appears to vary. Under identical conditions, ultrasound at 67 kHz was effective against Gram-negative *E. coli* and *P. aeruginosa* cultures but not against Gram-positive *S. aureus* and *S. epidermidis* cultures [92]. However, *S. epider-
midis responded favourably at a lower frequency (28.48 kHz) and a longer treatment time [88]. A variety of frequencies ranging from 28.48 kHz to 10 MHz has been utilised on bacterial biofilms [91, 93, 94]. A comparison between frequencies (70 kHz, 500 kHz, 2.25 MHz and 10 MHz) shows that lower frequencies appear more effective than higher ones [93, 95]. In both continuous and pulse ultrasound modes, high power intensities (200 mW/cm$^2$) appear more efficacious than low power intensities (2 and 20 mW/cm$^2$) [95, 96].

Most studies describe the effect of ultrasound on bacterial viability in terms of the changes in colony forming unit (CFU) per unit area. Although this provides an indication as to changes in the number of bacterial biofilm cells, it cannot measure physical changes in biofilms. Using ultrasound, one study suggests that bioacoustical affect biofilms [97], while another suggests it affects planktonic bacteria rather than biofilms [92]. It has also been suggested that ultrasound also enhances the transport of antimicrobial agents to bacteria [98], increases the transport of oxygen and nutrient to the cell and waste product away from the cell increasing bacterial growth rate [99], disturbs the cations in biofilms [100], and increases the permeability of the cell membrane [101-103].

Continuous [104] and pulsed [105] ultrasound at a frequency of 1 MHz with power intensities of 1 and 0.5 W/cm$^2$ for maxillary and frontal sinuses respectively have been evaluated in CRS treatment. Continuous ultrasound is more likely to cause a thermal effect [105], however, no thermal effect has been observed with pulsed ultrasound. In acute rhinosinusitis the efficacy of ultrasound is comparable to antibiotics and when compared to antibiotics was also the preferred treatment option [106]. Therapeutic ultrasound is also effective in treating CRS [86, 104, 105, 107, 108].

4.2.2. Bioelectric effect

In the last decade the influence of high and low intensity electric fields and current densities on biofilms has been studied. High intensity electric fields influence the organization of biological membranes [109], membrane analogues [110], the shape of the cell [111], cell behaviour [112] and the dimensions of the bacterial glycocalyx [113]. However, low electrical current has been shown to enhance the efficacy of antimicrobial agents against *P. aeruginosa* [114], *S. epidermidis* [114], *Streptococcus gordonii* [115], *E. coli* [116] and *S. aureus* [114] biofilms. This phenomenon has been referred to as the bioelectric effect.

Several methodologies of bioelectric effect on bacterial biofilms have been investigated, including direct current [117-119], radio frequency [116] and electromagnetic pulses [120]. The mechanism(s) of bioelectric actions on bacterial biofilms is not understood yet. A number of hypotheses have been proposed to explain the action(s) on biofilms [121]. These include increased bacterial growth due to electrolytic oxygen generation [122, 123], increased connective transport due to expansion and contraction of the biofilms [73], electrochemical generation of oxygen [119], and increased membrane permeability [124]. Most of the bioelectric studies on biofilms have analyzed the effects in terms of the changes in the number of CFU. Although this provides an indication of the effects on the number of biofilm cells, it does not measure chemical changes in biofilms including chemical bonds from covalent and hydrogen bonds, hydrophobic interactions, pH and EPS matrix. In addition, this analysis
does not explain physical changes in biofilms on either the nature of biofilms or attachment processes. Hence, it does not explain electrostatic charges, van der Waals forces, electron transportation or electromagnetic radiation.

The effects of application of a variety of currents ranging from 1 to 2000 mA and 15 µA/cm² to 9 mA/cm² have been investigated on biofilms [121]. The main variables in the use of bioelectric effects are electric field and current density. Although these variables have been thoroughly investigated on biofilms, their significance has not yet been established. In contrast, in the laboratory, the electric field is probably not the variable that breaks down the biofilms; however, it has been suggested that the current drives charged molecules and ions into the biofilm matrix [119]. Applying electric current alone has been reported as not killing biofilms [122, 124]; however, other studies have reported some electric current effects [123, 125].

Results have been analyzed in terms of changes in CFU with respect to time. Application of electric fields alone, have reduced the number of CFU of biofilms for a certain period of time, but they return to the original number of bacteria at the end of the treatment period [118, 119]. Time of treatment has been evaluated at 12, 24 and 48 hours [114, 124, 126]. When time was increased from 12 to 48 hours, electric current showed a slight increase in the viable count of biofilms compared with the absence of electric current [122]. A dual treatment by bioelectric effect and antibiotics showed a further reduction in biofilms beyond the use of antibiotics alone. The reduction is varied by a difference in magnitude ranging from 1-2 to 6-8 log [121]. However, variation in action on biofilms has shown different reactions according to type and concentration of antibiotics at the same level of electric field on the same species [114]. The morphology of biofilms could show the exact changes due to bioelectric effects if the bacterial biofilms behaviour were analysed by the changes of CFU in real time rather at a discrete point of time.

5. Summary

Ultrasound and bioelectric effect are new strategies for breaking down biofilms. However, their mechanisms are not well understood. Ultrasound frequencies that disturb bacterial biofilms need further investigation. The roles of time in the treatment and overall time of treatment have not been investigated and its effect with ultrasound has gained little attention. Bioelectric effect may disrupt bi-layer; however, the mechanisms through which electric currents or applied voltages cause a reduction in the number of viable biofilm cells is not conclusively understood. Evidence suggests that the biophysical approach could prevent the formation of biofilms and thus be clinically applicable to treat many chronic infectious diseases including chronic rhinosinusitis.

Several questions need to be answered. Are attachment bonds broken by ultrasound or does ultrasound disturb the biofilm structures? Is this caused by stretching the biofilm beyond the attachment forces or does the frequency affect the structural resonance of biofilms?
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References


