Motivation of Electroceutical Bandages for Treatment of Chronically Infected Wounds

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Abstract

In the United States, 6.5 million patients are affected by chronic wounds, sometimes complicated by infection. If the bacteria form a biofilm at the wound site, treatment of the infection becomes significantly more difficult. Biofilm bacteria are 500 to 5,000 times more resistant to antibiotic medications than the non-biofilm bacteria. Previous studies have shown that electric current enhances the activity of various antibiotics against biofilm-forming bacterial strains such as Pseudomonas aeruginosa and Staphylococcus epidermidis. This behavior has been referred to as the electro-bactericidal effect.

A large parametric investigation with various substrates, conductive patterns, and designs has led to a novel electroceutical bandage comprised of a silver-based ink on silk fabric, connected to a 6 V DC battery source and switch circuit for easy operation.

Currently, characterizing the electroceutical bandage includes in vitro tests using the bacterial strain Pseudomonas aeruginosa to test the efficacy of biofilm inhibition. The results have shown that our dressing successfully and repeatedly prevents the bacteria from forming a biofilm, as well as excludes bacteria from the anode of the bandage. It is noteworthy that use of an isolated electrode system i.e., electric field applied to the bacteria without direct flow of current through the bacterial layers, did not yield inhibition of the biofilm formation. Therefore, mechanistically, one may expect oxidation reactions at the anode to be important. This hypothesis is the subject of further on-going experiments. Further in vitro tests studying the effects of the bandage on already

established biofilms have been initiated as well. It is important to study both scenarios because this electroceutical bandage should prevent infection from developing at the wound site, as well as help treat existing infections. Severe biofilm infection can lead to amputation to prevent spread of infection. If more reliable and successful means of treating biofilm infections can be implemented, complications of chronic wounds will be reduced.

We have shown that engineered bandages with direct electric current flow between the wound-bandage interface inhibit bacterial growth at and around the anode. Due to this result, the conductive pattern design has been optimized to maximize this effect by increasing the surface area of the anode with respect to the available space on an average dressing of 5 cm x 5 cm. Currently, our measurements show a power density of 0.75 mW/cm2, well below the FDA limit of 0.25 W/cm2 for thermal burns therefore implying likely safe use of the dressing.

We hypothesize that the direct electric current is disrupting quorum sensing, or communication between the bacteria, effectively isolating them from each other due to oxidative stress at the anode. We believe this isolation prevents bacteria from forming a biofilm. The future experiments will focus on developing a more comprehensive understanding of the mechanisms behind biofilm inhibition in presence of low-magnitude direct currents.

Introduction

In the US, 6.5 million patients are affected by chronic wounds with an estimated $25 billion in healthcare costs and ~60% associated with bacterial biofilm infection (Sen et al., 2009). In the biofilm form, bacteria form ‘protective shields’ through extracellular polymeric substances (EPS) and become recalcitrant to antimicrobials and host immune defenses. While use of electrical interventions (primarily application of induced electric fields) on soft tissue dates back to the 1700s for enhanced cell migration for rapid wound healing, use of electrical interventions for infected wounds has been minimally implemented (Banerjee et al., 2015). In this thesis, a new engineered pre-clinical therapeutic bandage for treatment of *Pseudomonas aeruginosa* (PAO1) biofilms is presented by demonstrating both: (i) inhibition of biofilm formation and (ii) disruption of pre-formed biofilm *in vitro*.

Background

Millions of patients in the US are afflicted by chronic wounds. These wounds become much more dangerous when infected. If the infection develops into a biofilm infection, the bacteria become significantly more resistive to antibiotic treatment. Severely infected wounds can lead to amputation or even death. Alternate methods must be developed to attack biofilms infections in a robust and safe manner, to uphold the patient’s quality of life. This issue spans generations, races, and geographic location, and thus it is of high importance to generate new treatment methods.

Defining Biofilm Infections

There are two kinds of bacterial strains, (i) free-floating or planktonic and (ii) attached or sessile bacteria. A biofilm consists of bacteria attached to a surface. A bacterial biofilm is defined as "a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface," (Costerton, Stewart, & Greenberg, 1999). The polymeric matrix is connected with strong chemical bonds (Proal, 2008), resistant to environmental changes. Common biofilm-forming bacteria include *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*, both of which are commonly present in water, air, soil, and skin (Stewart & William Costerton, 2001). According to the Center for Biofilm Engineering, biofilm forms when bacteria adhere to surfaces in moist environments by excreting a slimy, glue-like substance. This slimy excretion is referred to as the extracellular polymeric substance (EPS) which holds the bacteria in the biofilm matrix. The bacteria form a biofilm, as depicted in Figure 1, in three phases: attachment, growth, and dispersal.

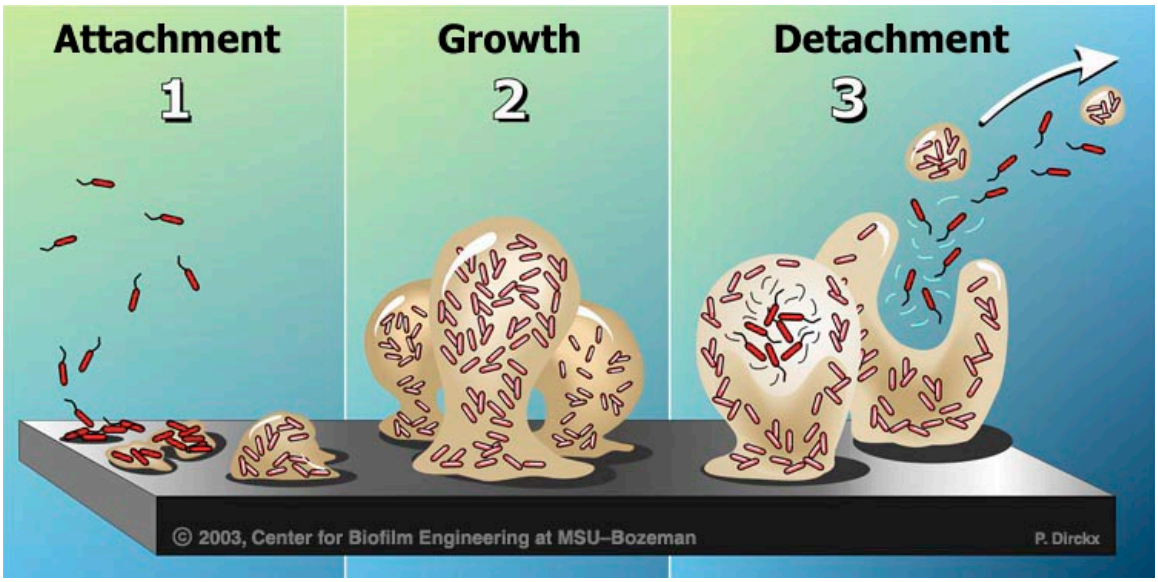

Figure 1: Depiction of the stages of biofilm formation: attachment, growth, and dispersal. (1) Planktonic bacteria encounter and attach to a moist surface and begin to produce their slimy extracellular polymeric substances (EPS). (2) When enough EPS is produced, the matrix is formed and the bacteria are now in a biofilm structure. This biofilm can form in a matter of hours. (3) The biofilm begins to spread in two forms of dispersal, (i) detachment of clumps of bacteria cells or (ii) "seeding dispersal" which releases individual bacteria cells into the environment. In the detachment phase, dispersed cells migrate to an area near the original biofilm and begin the cycle all over again. This allows the biofilm and multiply and spread. Figure used with permission from the Center for Biofilm Engineering at Montana State University.

A biofilm is a much more serious form of a bacterial infection because it is very resistant to any environmental changes, including antibiotic medications. The antibiotics in use today were created using studies of bacteria suspended in agar, or free-floating bacteria. However, is has been discovered in recent years that several bacteria preferentially attach to various substrates, both living and inert, and are highly adaptable organisms that exhibit survival skills in this form. Further, microbial biofilms are tolerant of antibiotic doses up to 1,000 times greater than those of planktonic bacteria (CBE). This field of research is rapidly expanding because biofilms cost the US billions of dollars

each year in energy losses, equipment damage, product contamination and medical infections (CBE).

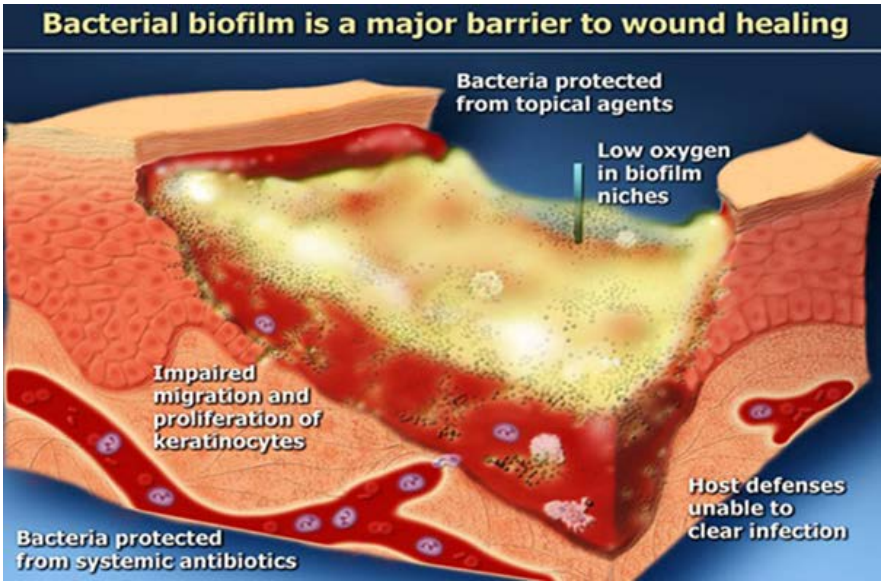

Figure 2: Structure of a biofilm shown in the context of a wound. Also shown are the host defenses of the body and surrounding tissue. Figure used with permission from the Center for Biofilm Engineering at Montana State University.

Treating Biofilm Infections

The behavior of biofilm in the presence of antibiotics had been characterized using a variety of bacteria strains, antibiotic treatments, and methods. In 2001, Mah and O'Toole suggested that various mechanisms are involved in treating biofilms with antibiotics, including physical or chemical barriers to the diffusion of antibiotics, nutrient limitation, and activation of the stress response of biofilm (Mah & O'Toole, 2001). They summarized that *Pseudomonas aeruginosa* biofilm acts as a diffusive barrier toward antibiotics and antimicrobials such as piperacillin and chlorine, however *Staphylococcus epidermidis* allow for the diffusion of rifampicin and vancomycin into the matrix (Mah & O'Toole, 2001). Antibiotics may seem to suppress the infection while medicated because

free-floating bacteria are killed off as exposed to the treatment. However, in most cases the antibiotics are not able to eradicate the biofilm, leading to recurrence of infection after treatment (Stewart & William Costerton, 2001). Further, thickness of the biofilm membrane impacted the effectiveness of antibiotic treatment. Hydrogen peroxide was able to penetrate a “thin” biofilm of average cell density on the order of 3.5 log colony-forming units (CFU) cm^{-2}, but not able to penetrate “thick” biofilm of average cell density on the order of 7.6 log CFU cm^{-2} (Mah & O'Toole, 2001).

It has been hypothesized in various publications that once exposing the biofilm to a direct electric current, the extracellular communication between bacteria is disrupted and antibiotics treatment becomes effective.

Methods

In this chapter, the design of the electroceutical dressings is described in detail, beginning with the original design inherited at the start of this Master’s project. The inherited design is then dissected to evaluate the various parameters of the wound dressing and optimize the design to create a more robust and repeatable bandage. Lastly, the fabrication of the optimized design is discussed in detail.

Inherited Design

The Department of Mechanical and Aerospace Engineering was brought onto this project with the Department of Surgery for designing and producing bandages that would exclude bacteria in a wound and inhibit biofilm infection for better wound healing outcomes. At the start of this Master’s project, a legacy design was used in previous

testing. However, the mechanisms involved in excluding bacteria were not understood and a detailed parametric study was needed to make sense of the rudimentary design. The inherited bandage design is shown in Figure 3, along with the 6V battery pack used to supply power to the system during *in vitro* and *in vivo* testing.

Figure 3: Inherited bandage including 6V battery pack. Bandage is comprised of a polyester fabric facing the wound, conductive circuit, cotton padding to absorb wound exudate, and an adhesive backing to seal the dressing. Notice the bandage and battery pack are connected by joining aluminum tape leads.

Enclosed in the battery pack were two 3V button cell batteries (Panasonic CR2450) connected by aluminum tape. The dressing was comprised of a polyester fabric with polyaniline pattern deposited on the outside and an aluminum tape circuit on the inside. The backing of the dressing was constructed from strips of an elastic adhesive wound dressing to protect the conductive circuit.

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