Methylene Blue Incorporated Polydimethylsiloxane to Combat Biofilms

Literature Review

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Introduction

Despite technological advances, there has been an increasing amount of Hospital-Acquired Infections (HAIs) in recent years, proposing a major concern to the medical community. Because of their increasing prevalence, HAIs are leading to increased death rates and medical costs. According to the U.S. Center for Disease Control and Prevention, about 1 in 25 hospital patients acquires at least 1 HAI, which is equivalent to 722,000 infections in the U.S. in 2011. These infections result in 75,000 deaths during hospitalization, costing the U.S. government approx. $35-$45 billion per year [1, 2]. More specifically, 60-70% of infections in hospitals can be attributed to medical device-related infections, especially in critically ill patients [3, 4]. Research to develop a novel solution to implant infections is ongoing. Some solutions include antibiotic coatings, silver nanoparticle coatings, nanostructured coatings, and photodynamic systems [5].

The field of photodynamic therapy (PDT) is a promising alternative to antibiotics, antimicrobial surfaces, and other current treatments because pathogens are unable to adapt to damage caused by PDT methods. Antimicrobial photodynamic therapy is based on the use of light-activated photosensitizers, which produce reactive oxygen species (ROS) when irradiated, to attack microbes through the oxidation of vital cell components. Bacterial adaptations to this type of oxidative stress is highly unlikely because, unlike antibiotics which inhibit a certain enzyme or protein, PDT targets all outer cellular components, inflicting irreversible damage. [5] However, a problem arises
in PDT from the lack of an efficient light source to activate photosensitizers as current models are expensive lasers possessing limited illumination areas.

**Overview of Biofilms**

A biofilm is a diverse colony of cells residing in a layer of extracellular polymeric substance (EPS), which is a structure composed of insoluble polysaccharides, proteins, and other molecules such as DNA or lipids. A biofilm typically hosts large communities of potentially infectious bacteria, fungi, and other pathogens. Within the biofilm is a network of channels responsible for the transportation of nutrients to cells. As a result of the intricate composition of a biofilm, bacteria within a biofilm possess a higher ability to resist antimicrobial drugs than planktonic, free floating bacteria [6].

Because biofilm forming bacteria adhere to static surfaces, biofilms make up for a large portion of medical implant related complications [6]. All medical implants are susceptible to biofilm formation as their surfaces cannot shed or combat biofilm attachment. Bacterial biofilms have been found to adhere and develop on a wide spectrum of medical implants including heart valves, dental implants, catheters, heart assistive devices, contact lenses, cerebrospinal fluid shunts, and artificial pumps [3, 4, 7, 8]. Biofilm formation on medical implants poses a serious threat to patient health as biofilms can lead to tissue damage, dysfunction of implant, and systemic dissemination of the infected area [9]. In fact, 60-70% of infections in hospitals can be attributed to medical device related infections, especially in critically ill patients [3, 9]. The main bacterial strains responsible for these incidents are the gram-positive *S. aureus*, *S. epidermidis*, and *S. viridians* and the gram-negative *E. coli*, *P. mirabilis*, and *P.*
*Pseudomonas aeruginosa* bacterial strains [5]. Because biofilm cells reside in a fortified microenvironment, indwelling cells have a high resistance to the immune response and antibiotic medication, making medical implant related infections extremely difficult to treat [6]. More specifically, the biofilm matrix limits antibiotic efficacy, meaning antibiotics alone are not enough to treat a biofilm. Eliminating a medical device related infection requires surgery and prolonged antibiotic use, both of which may have adverse effects on the patient. As a result, many research efforts are focused on reducing medical implant infections, including the development of antibiotic coatings, silver nanoparticle coatings, nanostructured coatings, and photodynamic coatings [5].

**Biofilm growth cycle**

Because biofilms are medically significant, accounting for over 80% of bacterial infections within the body, understanding the mechanism of biofilm development is important [5]. The mechanism for biofilm formation on medical devices can be divided into 3 stages: initial attachment, aggregation and maturation, and dispersal of cells [6]. Initial attachment, which can occur on nearly all medical implant surfaces, is the quick adhesion of cells to the surface of the implant [5]. During this interaction, van der Waal's forces and hydrophobic interactions allow for the bacteria to adhere to the implant [6]. There are also several bacterial membrane-bound proteins which contribute to this adhesion [5]. After attachment, the bacterial cells anchor themselves to the surface, start to produce extracellular polymeric substances, and colonize the surface of the medical implant [6]. The polysaccharide antigen known as polysaccharide intercellular adhesin (PIA) promotes intercellular adhesion and microcolony organization within a
biofilm while the EPS matrix serves as an outer structure and barrier to provide indwelling microbes with more favorable living conditions [5]. Following EPS production, small stable microcolonies begin to form, and microbes proliferate to form several layers of cells on the surface of the implant [5]. The EPS enables the maturing biofilm to develop into a complex three-dimensional structure composed of macro-colonies of bacteria connected by channels, which allows colonies to share water, signaling molecules, and nutrients [5]. During maturation, chemical signal molecules released from the bacteria into the EPS allow for quorum sensing, or the regulation of cell density and gene expression within a biofilm [6]. Quorum sensing also serves to determine the timing of cell attachment and detachment from the biofilm [5]. Once the biofilm matures, large clumps of cells detach and adhere to other surfaces or biofilms away from the original community [6].

Figure 1. Illustrated are the 3 stages of biofilm formation. Graphic was adapted from Montana State University [6].
Photodynamic Therapy

Photodynamic Therapy is based on the concept of photodynamic inactivation (PDI) of microbes through exposure to singlet oxygen or reactive oxygen species (ROS). PDT is an antimicrobial strategy proven to be effective against a broad range of pathogens, including those which have mutated to possess high levels of resistance to conventional antimicrobial drugs or those capable of forming biofilms [13]. PDI works through the activation of a dye called a photosensitizer (PS) with an adequate light wavelength in the presence of molecular oxygen [13]. Upon irradiation, the PS transitions from its lowest energy level (ground singlet state) to an excited singlet state, which can be converted to the excited triplet state. In the presence of oxygen, the excited triplet PS can undergo one of two possible chemical reactions: the type I mechanism which transfers electrons to form reactive oxygen species (ROS such as H$_2$O$_2$) or the type II mechanism which involves the energy transfer of a ground state triplet oxygen to produce highly reactive singlet oxygen (O$_2$) [13]. This mechanism is illustrated in Figure 2. Reactive oxygen species released from the PS then oxidizes bacterial biomolecules such as the lipids and proteins of the external structure of the microorganism, cytoplasmic membrane, cell walls, capsids, and lipid envelopes [14]. Through ROS induced oxidative stress, irreversible damage is inflicted on vital cellular components, causing the inactivation of microbes [13]. Photoresistant strains are highly unlikely to emerge as ROSs exhibit multiple mechanisms of attack as opposed to the single mechanisms exhibited by current antibiotics [13].
Figure 2. Illustration of the mechanisms of reactive oxygen species (ROS) produced during photodynamic action [13].

There have been considerable advances in the field of phototherapy, mainly in the assessment of new molecular dyes used for PDI. Many classes of PSs, such as phenothiazinium dyes, psoralens, perylenequinonoid pigments, natural and synthetic based tetrapyrrolic macrocycles, and fullerenes demonstrate strong antimicrobial properties [6]. Considering the colonization of catheters by microorganisms and the ease of incorporation of PSs into silicone-based materials, Wilson et al. developed antimicrobial catheter materials based on PDMS (polydimethylsiloxane), methylene blue (PS), and 2nm gold nanoparticles [18]. Testing the materials' photoefficacy on the gram-positive methicillin-resistant S. aureus, and the gram-negative E. coli, Wilson et al. found the materials to have a 1.0 log reduction of E. coli after 5 minutes of irradiation [18]. However, Wilson et al.'s research and similar research have been conducted using a professional Periowave 660 nm 250 mW diode laser which works in theory but presents many problems when applied in a medical setting [10, 11]. The first of which is that this laser is costly: a handheld laser system can cost as much as $3500 USD [17]. The second problem is the area of irradiation from the laser is too small to effectively...
treat biofilms on most medical implants [10]. Other research has found that the inactivation of bacterial strains is highly dependent on the structure of the PS being utilized [13]. Specifically, neutral, cationic, or anionic PSs can inactivate gram-positive bacteria, but cationic PS is needed to inactivate gram-negative bacteria [13]. This is because gram-positive bacteria are porous, while gram-negative bacteria are composed of negatively charged lipopolysaccharides, phospholipids, and lipoproteins [13].

**Figure 3.** Schematic representation of the cellular envelope of Gram-Positive (left) and Gram-Negative (right) bacteria [14].

**Conclusion**

From the statistics of medical implant related infections, it is evident that a new treatment for these infections must be developed as the current treatment method is expensive and can have adverse effects on the patient [5]. Surgeries and prolonged antibiotic therapies required for the removal of such infections are both ineffective, as the bacteria could develop resistance to the drug, and invasive. Photodynamic therapy offers a potential solution for medical implant related infections due to its ease of incorporation into various medical materials, such as PDMS and other silicones, and
non-invasive treatment method. The problem with PDT, however, is that light sources currently used for clinical trials are costly and ineffective for real scale implants.

Works Cited


