Biochemical and Biophysical Research Communications 438 (2013) 691–696

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Bacterial adhesion and growth reduction by novel rubber-derived oligomers

Hope T. Badawy [a], Pamela Pasetto [b], Jean-Luc Mouget [c], Jean-François Pilard [b], Teresa J. Cutright [d], Amy Milsted [a,*]

[a] Biology Department, University of Akron, Akron, OH 44325, USA
[b] Institut des Molécules et Matériaux du Mans, Université du Maine, Le Mans 72085, France
[c] MMS-Mer, Molécules-Santé, FR CNRS 3473 IUML, Université du Maine, Le Mans 72085, France
[d] Department of Civil Engineering, University of Akron, Akron, OH 44325, USA

ARTICLE INFO

Article history:
Received 10 July 2013
Available online 3 August 2013

Keywords:
Pseudomonas aeruginosa
Staphylococcus aureus
Staphylococcus epidermidis
Polyisoprene
Antibacterial
Rubber

ABSTRACT

In the medical field, attached bacteria can cause infections associated with catheters, incisions, burns, and medical implants especially in immunocompromised patients. The problem is exacerbated by the fact that attached bacteria are ~1000 times more resistant to antibiotics than planktonic cells. The rapid spread of antibiotic resistance in these and other organisms has led to a significant need to find new methods for preventing bacterial attachment. The goal of this research was to evaluate the effectiveness of novel polymer coatings to prevent the attachment of three medically relevant bacteria. Tests were conducted with *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* for oligomers derived from modifications of natural rubber (*cis* 1,4-polyisoprene). The different oligomers were: PP04, with no quaternary ammonium (QA); MV067, one QA; PP06, three QA groups. In almost all experiments, cell attachment was inhibited to various extents as long as the oligomers were used. PP06 was the most effective as it decreased the planktonic cell numbers by at least 50% for all bacteria. Differences between species sensitivity were also observed. *P. aeruginosa* was the most resistant bacteria tested, *S. aureus*, the most sensitive. Further experiments are required to understand the full extent and mode of the antimicrobial properties of these surfaces.

1. Introduction

Biofilms are communities of microorganisms growing in a self-made extracellular matrix of polysaccharides, DNA and other compounds. Generally, bacteria attach irreversibly to surfaces, and express different gene profiles in a biofilm compared to those of free living, planktonic bacteria. Once a biofilm has formed, the bacteria are extremely resistant to treatment with antimicrobials. A major problem is biofilms that grow on catheters, incisions, burns, medical implants and in immunocompromised patients [1]. It has been estimated that 80% of all microbial infections are associated with biofilms [2]. Nosocomial infections such as these present a major health burden. Reports have shown that bacteria in a biofilm are up to 1000-fold more resistant to the effects of antibiotics than bacteria growing as planktonic forms [3,4]. It is believed that the bacteria undergo unique phenotypic transformations that confer resistance to conventional therapeutic antibiotics [5]. The rapid spread of antibiotic resistance in these and other organisms mean that there is a great need for other methodologies of fighting bacterial infections, such as altering surfaces where biofilms form.

The three most prevalent bacterial species that develop biofilms causing infections are *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*. *S. aureus* has been identified as the dominant strain associated with biofilms in patients with chronic rhinosinusitis [6]. *S. aureus* and *S. epidermidis* are the two Gram positive strains most commonly associated with infections on medical devices such as catheters, orthopedics, artificial heart valves and shunts [7–12]. *P. aeruginosa* is a Gram negative rod commonly found as aggregates in the lungs of cystic fibrosis patients, as well as on the bandages of burn patients, on contact lens, urinary catheters, ports for cancer patients, and other implanted devices [13–18].

The initial attachment of bacteria to either biotic and abiotic/implanted surfaces can increase their resistance to the natural immune responses of the body and to antibiotic treatments. Most attachment occurs relatively quickly, sometimes in as little as 60 s [19,20]. Although attachment is rapid, there are distinct steps

* Corresponding author.
E-mail addresses: hope.badawy@gmail.com (H.T. Badawy), pamela.pasetto@univ-lemans.fr (P. Pasetto), jean-luc.mouget@univ-lemans.fr (J.-L. Mouget), Jean-Francois.Pilard@univ-lemans.fr (J.-F. Pilard), tcutrig@uakron.edu (T.J. Cutright), milsted@uakron.edu (A. Milsted).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.07.120

involved in the colonization of bacteria to a surface: the conditioning of the surface via accumulation of organic molecules, a reversible binding stage of cells, followed by irreversible attachment and biofilm development. The first major step is the reversible adherence of single cells to a surface by van der Waals forces and hydrogen bonding. The second step is less likely to be reversible and is mediated by specific interactions between cells and host proteins [21]. Rohde et al. [1] found that infections caused by *S. epidermidis* were dependent on the organism's ability to adhere to surfaces prior to assembling into a large biofilm. Both attachment steps occur by a variety of mechanisms including specific receptors, proteins and overall surface charge, which leads to the extensive hydrogel-like extracellular matrix. While the exact mechanism of biofilm formation in terms of specific receptor or other proteins is species dependent, the most effective method of preventing biofilm formation is still to prevent the initial stages of protein and bacterial attachment [5,7,22,23]. Studies have shown that surface charge, hydrophobicity, surface roughness and microtopography can be important in preventing the initial attachment, as well as culture conditions, pH, ionic strength and the species of bacteria involved [24].

We have developed novel polymer formulations derived from natural rubber [25]. The overall goal of this research is to evaluate the effectiveness of the novel polymers in preventing attachment and subsequent biofilm formation of three medically relevant bacteria. The antimicrobial properties of the acrylate and ammonium oligomers are also compared.

2. Materials and methods

2.1. Bacterial cultures

Pseudomonas aeruginosa PA01 (ATCC # BAA-47), *Staphylococcus aureus* FDA 209 (ATCC # 6538), and *Staphylococcus epidermidis* RP62A (ATCC # 35984) were the medically relevant organisms chosen for this study. All strains were purchased from the American Type Culture Collection and maintained on Tryptic Soy Agar (TSA). Stock cultures were grown overnight at 37 °C in Tryptic Soy Broth (TSB). For attachment assays, cultures were inoculated with cells of OD_{600} of approximately 0.05, corresponding to a starting inoculum of 10^7 cells/mL, as detailed below.

2.2. Growth curves

Growth curves were performed for each strain to ascertain the initial inoculation density for the static attachment assays (Fig. 2). They were also used to access the toxicity of the polymers by comparing initial and final microbial numbers. One mL of overnight culture was transferred into 100 mL of fresh TSB medium and incubated at 37 °C and 250 rpm. Initial OD_{600} and cell counts were carried out at time zero. A one mL sample was removed every hour for 13 h, and an OD_{600} taken. Cells were then diluted, plated on TSA plates using a spiral plater (Autoplate® Spiral biotech, Bethesda, MD, USA), incubated overnight at 37 °C and colonies counted to determine colony forming units, CFU/ml.

2.3. Preparation of polymer surfaces

The syntheses of all the oligomers used in this study have been described in detail [25]. Three different types of oligomers were prepared (Fig. 1: PP04, MV067, PP06) and combined in different proportions (Table 1) to generate the different surfaces. In addition to the acrylate oligomer composed of the simple polyisoprene chain (PP04, Fig. 1) [26], we introduced one quaternary ammonium (QA) group at one chain end (MV067, Fig. 1), or three QA groups in the same oligomer (PP06, Fig. 1). A series of formulations was prepared varying the percentages of the different oligomers. The rationale was to start with 100% of the simple acrylate oligomer, which is expected to have the lowest or no antibacterial action, and to increase the weight percent of a second oligomer containing one or three QA groups, to evaluate QA action and to optimize the composition of the coatings. In some cases HDDA (hexanediol diacrylate), a reactive diluent, was added to reduce pre-polymerization solution viscosity. For each coating, the prepolymerization mixture was applied into each well of a 24 well plate and polymerized [25].

2.4. Assaying antibacterial effects of polymers

Growth surfaces of 24-well tissue culture plates were coated with mixtures of polymers, as specified in Table 1. Each plate consisted of a positive and negative control and replicates of each strain tested. Table 1 lists the testing protocol. One mL of TSB was added to each well of the plates. Two μL (1:100 dilution of overnight culture of approximately $OD_{600} = 0.05$) of *P. aeruginosa*, *S. aureus*, or *S. epidermidis* were introduced into separate sample wells and incubated at 37 °C for 3 h. Triplicates of controls and samples were used for each bacteria-polymer sample.

The amount of attachment on each polymer surface, as well as planktonic growth (unattached cells in the medium overlying the surface), was assayed at the end of the contact time, by two methods. The first method used 1 μL of growth medium solution for colony counts (CFU/ml), as described in Section 2.2. The second method assessed the amount of loosely attached biofilm. Two mL of fresh TSB medium was added to the washed plates, the wells were sonicated for 8 s, and medium containing the detached cells was removed, diluted and plated on TSA plates, incubated at 37 °C overnight, and colonies counted to obtain CFU/ml of surviving bacteria.

2.5. Statistical analysis

Statistical significance was assessed by a 2-tailed pairwise comparison *t*-test between the control and sample of each bacteria-polymer combination. Separate statistical analyses were conducted for CFU on the polymer surface and in the medium. Significance was assigned when $p \leqslant 0.05$. Two-tailed comparisons with $p \leqslant 0.001$ were considered highly significant. Error bars in graphs represent standard error.

3. Results and discussion

The present study was designed to evaluate the antimicrobial properties in biological settings for three different oligomers: PP04 (no QA), MV067 (one QA), PP06 (3 QAs) (Fig. 1). Two series of attachment assays were performed. The first series used surfaces prepared from a mixture of two oligomers, PP04 and PP06 in various proportions, with or without addition of HDDA. The experimental protocol in terms of proportions of each polymer are listed in Table 1. Each separate surface, composed of different proportions of the oligomers, was given a unique identifier, formulation S1, S3, etc., (Table 1). HDDA is used to create a smoother surface. This allowed us to compare the anti-attachment properties of coatings differing by the proportions of oligomers, and estimating a possible influence of the surface structure on cell growth and attachment. The second series of experiments was designed to compare the attachment properties of surfaces made from acrylate alone (PP04), from oligomer containing one QA (MV067), or a mixture of the two oligomers. For these surfaces HDDA was not added.

Figs. 3 and 4 present results as growth relative to control, on the control or polymer surface and in the growth medium above the