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Antibacterial activity on superhydrophobic titania nanotube arrays

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ABSTRACT

Bacterial infections are a serious issue for many implanted medical devices. Infections occur when bacteria colonize the surface of an implant and form a biofilm, a barrier which protects the bacterial colony from antibiotic treatments. Further, the anti-bacterial treatments must also be tailored to the specific bacteria that is causing the infection. The inherent protection of bacteria in the biofilm, differences in bacteria species (gram-positive vs. gram-negative), and the rise of antibiotic-resistant strains of bacteria makes device-acquired infections difficult to treat. Recent research has focused on reducing biofilm formation on medical devices by modifying implant surfaces. Proposed methods have included antibacterial surface coatings, release of antibacterial drugs from surfaces, and materials which promote the adhesion of non-pathogenic bacteria. However, no approach has proven successful in repelling both gram-positive and gram-negative bacteria. In this study, we have evaluated the ability of superhydrophobic surfaces to reduce bacteria adhesion regardless of whether the bacteria are gram-positive or gram-negative. Although superhydrophobic surfaces did not repel bacteria completely, they had minimal bacteria attached after 24 h and more importantly no biofilm formation was observed.

1. Introduction

Bacterial infections are a serious issue for many implanted medical devices. Infections of mechanical heart valves, pacemakers, heart assist devices, etc. are associated with high rates of patient mortality [1,2]. It is estimated that more than half of the hospital-acquired infections in the United States come from biofilms. Biofilms are protective films composed primarily of proteins and polysaccharides created by the bacteria [3,4]. The biofilm protects the bacteria by blocking antibiotics, making these infections difficult to combat [5–10]. Additionally, the overuse of antibiotics is also giving rise to bacteria strains which are resistant to common drugs [6,9]. Infectious bacteria are commonly divided into two categories based on the composition of their cell walls: gram-positive and gram-negative [5,9–12]. Gram-positive bacteria have a cell wall with a layer of peptidoglycan that is typically 15–80 nm thick [12]. The peptidoglycan layer in the cell wall of a gram-negative bacterium is typically 1–2 nm [12]. Common gram-negative bacteria include *Escherichia coli* and *Pseudomonas aeruginosa*, the latter of which is often studied because of its association with device-related infections [6]. *Staphylococcus aureus* is a gram-positive bacteria that is found naturally on human skin, and is also commonly associated with device-related infections [3,13,14]. Because these different types of bacteria must be treated with different medications, recent work has focused on modifying the surfaces of implantable medical devices that can reduce adhesion of both gram-positive and gram-negative bacteria [1].

Different methods of surface modification have been investigated for their potential to reduce medical device-associated infections. A common approach is to modify the surface with antibacterial coatings [1,15]. Other approaches have involved doping materials with silver ions. Silver has been shown to have antibacterial properties, but these surfaces have the same limitations as other antibacterial coatings because the silver ions will eventually diffuse out [5]. Some studies have also looked into using photocatalytic materials that have antibacterial properties when exposed to UV light, but these materials have poor long-term affinities towards organic molecules [10,11,16]. Another approach has been to promote the adhesion of nonpathogenic bacteria over more pathogenic bacteria, but there have been few successes with this approach [1]. To avoid the issues of these approaches, recent studies have looked for ways to prevent the initial attachment of bacteria. One such approach is to employ superhydrophobic surfaces, which

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display a contact angle greater than 150° and roll-off angle (i.e., the minimum tilt angle of the surface at which liquid droplets starts to roll off from the surface) less than 10° for water. These differ from superhydrophilic surfaces, which are those that display very low (typically < 10°) contact angles.

Superhydrophobic surfaces are being investigated for their anti-biofouling properties because the low solid surface energies of these surfaces reduce the adhesion of contaminants and water, making them easy to clean [17–20]. These surfaces are typically fabricated either by covalently attaching molecules with low surface energies to a roughened surface or by roughening the surface of a material which is already hydrophobic [7,17,21,22]. Such surfaces are of interest for a diverse array of applications. For example, superhydrophobic surfaces have been used to reduce the attachment of marine organisms to ship hulls and reduce the drag forces within pipes.[23] They have also been used to create stain-resistant fabrics [23]. As another example, superhydrophobic surfaces have been used to create miniaturized labs for performing biological tests [24–26]. They have also been used to improve the efficiency of turbines and steam engines by reducing the buildup of water [23]. Some previous reports show that superhydrophobic surfaces tend to reduce the attachment of a range of bacteria strains, but other reports show attachment and biofilm formation, indicating that more investigation is needed [5,9,18,27–30]. Further, superhydrophobic surfaces exhibit reduced protein adsorption and any proteins that do adhere are easy to remove [11,31]. These properties are expected to make it more difficult for bacteria to attach and form biofilms on superhydrophobic surfaces.

In this study, we have investigated the adhesion of *S. aureus* and *P. aeruginosa* to superhydrophobic and superhydrophilic titania nanotube arrays. Titanium was chosen as a base material due to its common use in many implantable medical devices. The different surfaces were fabricated by first anodizing and chemically etching titanium to form titania nanotube arrays. The titania nanotube arrays were then silanized to modify the surface chemistry and induce superhydrophobicity or superhydrophilicity. The surfaces were characterized using scanning electron microscopy (SEM) to determine surface topography, contact angle goniometry to determine surface wettability, X-ray photoelectron spectroscopy (XPS) to characterize surface chemistry, and X-ray diffraction (XRD) to examine the crystal structures. *S. aureus* and *P. aeruginosa* adhesion to different surfaces was measured using fluorescence microscopy and SEM, after 6 h and 24 h of culture. The results showed that fewer bacteria attached to the superhydrophobic surfaces when compared to the superhydrophilic and control surfaces. Further, the superhydrophilic surfaces did not show significant differences in the number of attached bacteria when compared to the unmodified titania nanotube arrays.

2. Materials and methods

2.1. Fabrication of titania nanotube arrays

Titania nanotube arrays were fabricated from titanium sheets (0.1 cm thick) cut into 2.5 × 2.5 cm squares. The titania nanotube arrays were fabricated using the anodization process described elsewhere [32–36]. The titanium sheet was used as the anode and platinum foil was used as the cathode. The electrolyte used for anodization was composed of 95% v/v diethylene glycol (DEG, Alfa) and 2% v/v hydrofluoric acid (HF, Alfa) by volume in de-ionized (DI) water. The anodization was done for 24 h at 60 V. After anodization, the titania nanotube arrays were rinsed three times with DI water, dried with nitrogen gas, and annealed for 3 h at 530 °C.

Superhydrophobic titania nanotube arrays were fabricated by modifying surfaces with chemical vapor deposition of (heptadecafluoro-1,1,2,2-tetrahydrodecyl)trichlorosilane (referred to as S1 in this manuscript, Gelest). The titania nanotube arrays were first etched with plasma at 200 V in 10 cm^3/min of oxygen gas for 10 min and then heated for 1 h at 120 °C with 150 μl of S1 in a closed chamber. The superhydrophobic titania nanotube arrays were then rinsed with DI water, dried, and stored until further use.

Superhydrophilic titania nanotube arrays were fabricated by modifying surfaces with poly-ethylene glycol 2-[methoxy(polyethyleneoxy)propyl]trimethoxysilane (referred to as S2, Gelest). As with the superhydrophobic arrays, the titania nanotube arrays were first etched with plasma at 200 V in 10 cm^3/min of oxygen gas for 10 min and then were placed in a 2 vol% of S2 in ethanol solution for 24 h [37]. The superhydrophilic titania nanotube arrays were then rinsed with DI water, dried, and stored until further use.

In this manuscript, the different surfaces are labeled as following: unmodified titanium, referred to as Ti; unmodified titania nanotube arrays, referred to as NT; superhydrophobic titania nanotube arrays, referred to NT-S1; and superhydrophilic titania nanotube arrays, referred to as NT-S2. The surfaces were sterilized in ethanol for 30 min, then rinsed with DI and dried before all biological experiments.

2.2. Surface characterization

The surface morphology of the titania nanotube arrays was characterized using a JEOL JSM-6500 field emission scanning electron microscope (SEM). The surfaces were coated with 10 nm of gold and imaged at 15 kV. The outer diameters and thicknesses of the nanotubes were measured using ImageJ.

The surface wettability of the different surfaces was characterized using the sessile water droplet method for measuring contact angles. The measurements were taken using a Ramé-Hart Model 250 goniometer connected to a camera. Approximately 10 μl water droplet was formed on a syringe and placed on the substrate surface. Water was added and removed from the droplet to measure the static, advancing and receding contact angles on the surface. Roll-off angles were measured by placing the droplet onto the surface and then tilting the goniometer. Images of the static contact angles were taken after the water droplet had been in contact with the surface for 10 s. Further, the solid surface energy was estimated using Owens-Wendt analysis (using a surface tension for water of 72.1 mN/m) [37,39].

The surface chemistry of the titania nanotube arrays was characterized using X-ray photoelectron spectroscopy (XPS). Survey spectra were collected along with high resolution spectra scans for titanium and oxygen. The survey spectra were conducted with a pass energy of 187.85 eV from 0 to 1100 eV, while the high-resolution spectra were obtained at a pass energy of 10 eV. The scans were completed using an ESCA Systems X-ray Photoelectron Spectrometer 5800 with a monochromatic Al-Kα-X-ray spot source at 1486.6 eV [38].

The crystal structure of titania nanotube arrays was characterized using X-ray diffraction (XRD). The scans were collected over a 2θ range of 20°–80° with θ = 1.5° [31]. They were run at a rate of 1 step per sec with a step size of 0.01°. DIFFRACT.EVA was used to filter the data and identify the relevant peaks.

2.3. Preparation of bacteria cultures

P. aeruginosa and *S. aureus* cultures were obtained from 10 ml tubes from bacteria solutions stored in glycerol (30% v/v, Sigma) at a concentration of 15% v/v and stored in a −80 °C freezer. The culture preparation steps are described elsewhere [6]. Prior to each study, one 10 ml tube was thawed at room temperature for approx-