Inhibition of Biofilm Formation with Electrospun Titanium Dioxide Films

Alyssa Abraham
Department of Chemistry and Chemical Engineering, Tagliatela College of Engineering

Abstract

A biofilm is a group of microorganisms that attach and grow on a solid surface. Biofilms are common in nature. They can cause clogs and corrode pipes, cause infections when formed on medical implants, and cost billions of dollars in damages, so it is desirable to prevent their growth. This work explores the ability of electrospun titanium dioxide to inhibit the growth of biofilms of *pseudomonas putida* compared to titanium dioxide films prepared by a sol-gel technique. Although the sol-gel method aided in the prevention of biofilms, the electrospun titanium dioxide slides showed less biofilm attachment.

Introduction

A biofilm is a group of microorganisms that grow on a solid surface. They can be characterized into groups by their structural heterogeneity and genetic diversity. Biofilms consist of many different species of bacteria and archaea in a matrix. The matrix protects the cells and allows communication between them through chemical and physics signals. Some biofilms have water channels that allow nutrients to be distributed.

Biofilm formation starts from a few microorganisms that attach to a surface through weak, reversible, van der Waals forces. These microorganisms are called colonists. They attach permanently through adhesion methods such as pili. The colonists provide a base for other cells to attach. Only a few species can attach to the surface on their own. Other cells must anchor to the matrix or to previous attached microorganisms instead. The biofilm grows as the cells divide and more bacteria adhere to it.

Biofilms are common in nature, usually found on substances that are in or near aqueous solutions. Many bacteria have mechanisms that allow them to adhere to different surfaces and other bacteria. There are good and bad types of biofilms. A good biofilm is used to clean and treat odorous air and remove harmful organic compounds from water. Sewage treatment plants use biofilms that have been grown on filters to remove and digest harmful organic compounds as the wastewater flows over them. Another form of biofilms is the biofilter. Biofilters treat odorous air. The bacteria use the odorous gases in the air as food for energy and nutrients. A biofilter can reduce odor in air by eighty-five percent.

Not all biofilms are useful, so in many cases it is desirable to prevent their growth. One of the most common biofilms is dental plaque. Dental plaque can build up on teeth and when not removed regularly can lead to dental caries, which can cause cavities. Other biofilms form on the interior of pipes where they can clog and corrode them. Biofilms can also be a medical hazard. They form on implanted tubes and wires, which can cause infections. Biofilms cost billions of dollars each year for equipment damage, contamination in products, loss of energy, and infections in patients [4].

A number of studies have demonstrated that titanium dioxide (TiO_2) nanoparticles form a good surface to prevent biofilm growth. TiO_2 aids in the inactivation of bacteria such as *E. coli*, *E. cloacae*, *P. aeroginosa*, and *S. typhinusium* in thin film applications [1]. In one study by Choi et al, TiO_2 was applied on an alumina support as a catalyst for wastewater treatment and it was found that there were fewer bacteria on the alumina support coated with TiO_2 as opposed to the one without the TiO_2 [3].

In another study by Ciston et al, the hydrophobicity of the TiO_2 coating was compared to uncoated surfaces see how the coatings affected the molecule adsorption on the membrane surface. It was found that the coatings had a higher hydrophilic surface. This shows that organic molecules would favor an uncoated membrane surface rather than the TiO_2 surface. The study showed that the coated discs had less than twelve percent of the bacterial attachment of the uncoated discs. The TiO_2 coating oxidizes the organic materials at the membrane surface. This prevents the adsorption of the bacteria to the surface.

Ciston et al also demonstrated the use of TiO_2 as a photocatalyst to reduce cell attachment. They synthesized TiO_2 by the sol-gel technique and showed that ultraviolet light and TiO_2 alone reduced cell attachment but when combined, produced a system that greatly diminished cell attachment [1].

This work explores the ability of electrospun titanium dioxide to inhibit the growth of biofilms of *pseudomonas putida* compared to titanium dioxide films prepared by a sol-gel technique.

Electrospinning is a technique used to produce nanofibers of polymers and other materials. Nanofibers are ultra-fine fibers that have small diameters, large surface areas, and small pore size. Conventional fiber spinning methods such as wet, dry, melt, and gel spinning form polymer fibers in the micrometer range. Electrospinning is unique and useful because it is capable of producing polymer fibers in the nanometer range. Nanofibers are produced by using an electrostatically-driven jet of polymer solution. A high voltage is applied to a syringe needle as a polymer solution is expelled from the syringe. The polymer solution becomes an electrified jet, which undergoes a whipping process. During this process the solvent evaporates, leaving the polymer fiber. The fiber is stretched and reduced in diameter then deposited on a grounded collecting metal plate.

A variety of different shaped fibers can be formed such as circular, branched, flat, and bent. The size and dimensions of the deposited fibers can be modified by changing the electrospinning parameters such as the applied voltage, distance between the syringe needle and collecting plate or the polymer solution concentration. To electrospin materials that are not polymers it is often necessary to mix the material with a polymer (called a carrier polymer) that can be electrospun. Then the polymer can be extracted or decomposed after the film has been deposited [2].

Materials and Methods

The TiO_2 was prepared in a solution with polyvinylpyrrolidone (PVP). PVP, 0.45 g, was dissolved in 7.5 mL ethanol. In another flask 3 mL acetic acid and 3 mL ethanol were mixed. Then 1.56 mL of titanium (IV) isopropoxide was added to the acetic acid/ethanol solution. This reaction was conducted under N2 (in a glove bag). After 10 minutes, the titanium (IV) isopropoxide solution was removed from the glove bag and combined with the PVP solution. The solution was then stirred on a stir plate for an hour. It was electrospun immediately. A volume of 0.05 mL of the solution was spun onto glass slides at a rate of 0.2 mL/h, 5 kV, and at a distance of 5 cm. The slides were then heat treated at 500 ^{o} C for 5 hours.

Pseudomonas putida was cultured in a Teknova M9 Minimal salt broth minus Leucine with 50 ug/mL Ampicillin. It was then placed in petri dishes with slides coated with electrospun titanium dioxide, sol-gel titanium dioxide, or no coating.

The samples were characterized by Infrared (IR) spectroscopy using a Nicolet 6700 FT-IR Spectrometer. A Rigaku MiniFlex II Desktop X-ray Diffractometer (XRD) was used to determine the structure of the crystal. An HU-70 Scanning Electron Microscope (SEM) was used to confirm the formation of fibers. A Leica Compound Microscope with camera was used to obtain images of the biofilms.

Results and Discussion

Titanium isopropoxide reacts with water to form titanium dioxide [5]:

$$Ti\{OCH(CH_3)_2\}_4 + 2\ H_2O \rightarrow TiO_2 + 4\ (CH_3)_2CHOH$$

Surfaces coated with electrospun TiO_2 were characterized prior to testing the surfaces for their ability to inhibit bacterial attachment and biofilm growth. Figure 1 shows an SEM image of the electrospun TiO_2 films after heat treatment to remove the PVP. The fibers have a diameter of 4.9 μm.

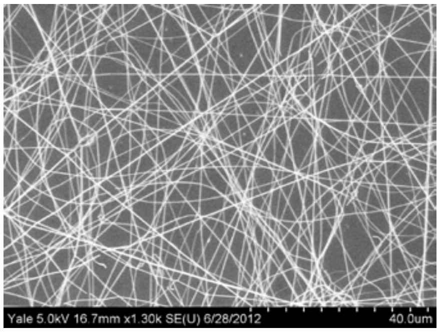

Figure 1. An SEM image of electrospun TiO_2 fibers after undergoing heat-treatment.

Characterization of the electrospun films before (Figure 2) and after (Figure 3) heat-treatment was done with IR as well. IR spectra show that there was no PVP on the slides after the heat treatment.

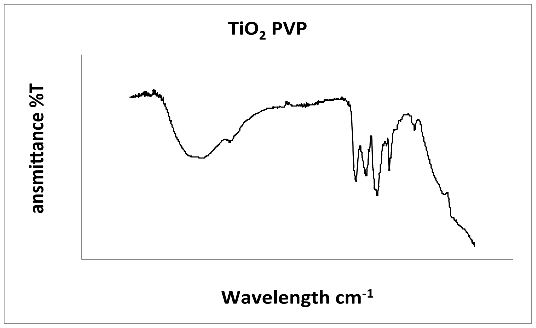

Figure 2. IR spectrum of TiO_2 PVP before heat-treatment.

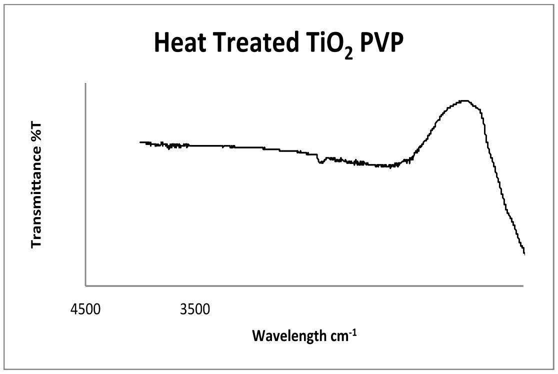

Figure 3. IR spectrum of TiO_2 PVP after heat-treatment showing only TiO_2.

X-ray diffraction patterns were also obtained for the electrospun fiber mats. Before heat treatment (Figure 4) the TiO_2 films were largely amorphous, possibly with small crystals of the anatase phase.

After heat treatment, the fibers were mostly anatase TiO_2, though a transformation to rutile was beginning (Figure 5).

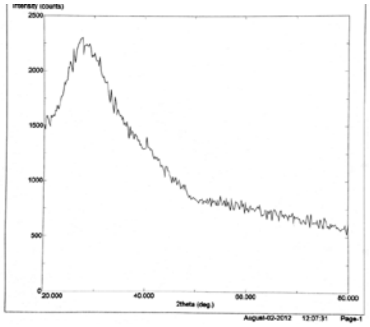

Figure 4. An XRD spectrum of TiO_2-PVP showing the films possibly with small crystals of the anatase phase.

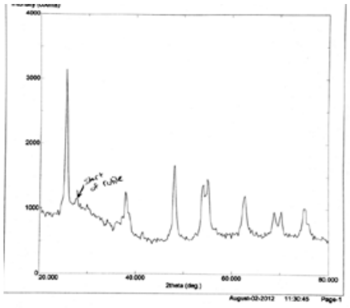

Figure 5. An XRD spectrum of heat-treated TiO_2 showing a transformation into the rutile phase.

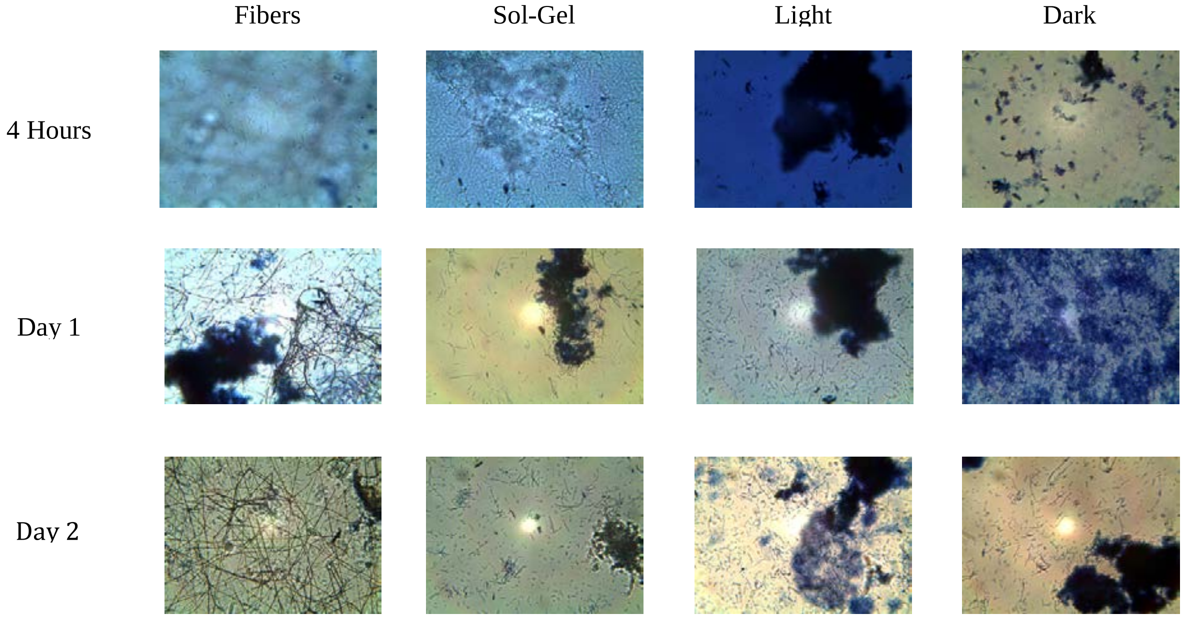

Figure 6. Representative images of each of the different types of slides at intervals observed. The first column shows the heat-treated fibers. The second column shows the heat-treated sol-gel method. The third column shows plain glass slides in the light. The fourth column shows plain glass slides in the dark.

Figure 6 shows representative optical images of slides after exposure to *p.putida* for varying lengths of time. The control of plain glass slides exposed to *p.putida* in the light showed less bacterial attachment compared to the control of plain glass slides in the dark. Preliminary results show that the slides containing the electrospun fibers inhibited the biofilm growth the most. The sol-gel method had less biofilms than the blank slides.

Conclusions

These preliminary experiments suggest that the slides coated with titanium dioxide fibers inhibit *p.putida* biofilm growth better than slides coated with sol-gel TiO_2 and plain glass slides. As expected, slides exposed to light have fewer attached bacterial and biofilms than those kept in the dark. Titanium dioxide generates hydrogen peroxide and hydroxyl radicals upon exposure to light which helps kill the bacteria. Regions of the coated glass slides with thicker films had less biofilm growth on them.

Experiments will be repeated and the numbers of live and dead cells quantified, potentially using live-dead staining techniques. Parameters such as film thickness, fiber diameter and heat treatment temperature will be varied and the effect on biofilm growth examined.

The method will then be tested in environments that are prone to biofilms such as sewers. The life of the electrospun titanium dioxide coatings will also be looked at to see how often they need to be reapplied.

References

1. Ciston, Shannon, Richard Lueptow, and Kimberly Gray. Bacterial attachment of reactive ceramic ultrafiltration membranes. *Journal of Membrane Science*. *320*. (2008): 101-07. Web. 1 Mar. 2012.
2. Frenot, Audrey, and Ioannis Chronakis. Polymer nanofibers assembled by electrospinning. *Current Opinion in Colloid and Interface Science 8*. (2003): 64-75. Web. 4 March 2012.
3. H. Choi, E. Stathatos, D.D. Dionysiou, Photocatalytic TiO2 films and membranes for the development of efficient wastewater treatment and reuse systems. *Desalination 202* (2007) 199–206.
4. Titanium isopropoxide. *Wikipedia*. Wikipedia, 21 2012. Web. 19 Oct 2012.
5. What Is Biofilm? *Bio news online*. Transgalactic Ltd, 2005. Web. 4 March 2012.

Biography

Alyssa Abraham is currently a junior at the University of New Haven majoring in Chemistry and Forensic Science. She hopes to go to graduate school to obtain a Ph.D. in Analytical Chemistry.

Alyssa spends her spare time participating in clubs and organizations on campus. She is a Resident Assistant, Secretary of the student chapter of the American Chemical Society, Vice President of the Biology Club and Pep Band and a member of the admissions team and marching band.

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