

SYNTHESIS OF AN ANTIMICROBIAL TEXTILE COATING

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**Abstract.**

A titania nanosol was synthesized and coated onto nylon/cotton blended textile substrates. The substrates were characterized via SEM for adhesion and nanoparticle formation, then subjected to antimicrobial efficacy tests. The titania nanosol was successfully coated on to textiles samples. Particles were observed to be around 2 by 3 micrometers and formed between the interstitial space of textile fibers. Although larger than typical nanoparticles, the coatings exhibited what seemed to be antimicrobial activity. Titania nanosol coated textile samples were subjected to Kirby Bauer Assay in the presence of *S. aureus*. The coated textile sample exhibited an inhibition of growth around its edges while the uncoated sample encouraged growth. A post-antibiotic effect was observed to be 1.2 hours on *S. aureus* when exposed to the titania coated textile.

## Introduction.

Antimicrobial coatings have been developed for a variety of different applications to reduce the proliferation of bacteria, fungi, and viruses on surfaces. Current scientific research involving antimicrobial coatings for textiles is gaining attention in the healthcare industry due to the increased risk of healthcare associated infections (HAIs) (Gouveia, 2010). Reducing the number of pathogenic microorganisms in a patient's environment is now a high priority in all healthcare institutions. Antimicrobial coated surfaces can potentially reduce these troublesome infections.

The goal of this project is to create an antimicrobial coating using nanoparticle titanium dioxide to be applied to textile samples. A titanium dioxide coating will be characterized and observed for antimicrobial activity. Titanium dioxide (also known as titania) was chosen as the base for this coating because of its photocatalytic activity. The photocatalytic activity of nanoparticle titania is useful for antimicrobial applications and is currently being investigated for its ability to inhibit the growth of pathogenic microorganisms (Dastjerdi, 2010). Partly due to the recent interest in reducing transmission of infectious disease, titania nanoparticles could be used for surface treatments to deter growth of pathogenic microorganisms in the surrounding clinical environment. Every year, 1.7 million HAIs cause almost 100,000 deaths in the United States (Pollack, 2010). Those that don't end in death can cause life-threatening illnesses. HAIs are generally caused by transmission of microbes between surfaces, patients and employees. Patients that are immune-suppressed are among the most susceptible to HAIs. In fact, any patient admitted into a healthcare facility for a medical transplant, disease, or critical trauma is at risk (Neely, 2000). One of the most common of these infections is *Staphylococcus aureus*, also known as "Staph." *S. aureus* is a facultative anaerobic gram positive coccus (spherical)

bacterium, which indicates its spherical shape and lack of an outer membrane. *Staph* colonizes in both aerobic and anaerobic conditions and is a normal flora in about 30% of industrialized countries' populations (Page, 2007). Many strains of *Staph* have the ability to develop a resistance to antibiotics. For example, methicillin resistant or multi-resistant *Staphylococcus aureus* (MRSA) was found in 50 % of *Staph* infection cases in United States ICUs in 2007 (Rosenthal, 2009). *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterococcus* have also developed antibiotic resistant strains that are difficult to control in clinical environments (Rosenthal, 2009). *Clostridium difficile* has become one of the leading causes of infection due to its spore forming bacterial cell wall. The dormant endospores of *C. diff.* allow it to undergo harsh conditions from antibiotics and antimicrobial surfaces (Pant, 2011). One of the reasons that these infections might find their way to immune deficient patients is contamination of the surrounding environment. For example, patients are in constant contact with bed linen during their hospital stay. The transfer of infectious disease through linen, although not highly recognized, is very common in clinical settings. Under certain temperatures and humidity, textiles such as bed linen are a perfect place for microbes to grow. (Gouveia, 2010).

In most healthcare facilities, linens, scrubs and gowns are rented out by an industrial launderer. During the transportation of linen from the industrial laundry plant, it may be handled by up to eight different people before it is placed on the bed of a patient (according to healthcare personnel at Sierra Vista Regional Medical Center in San Luis Obispo, CA). Environmental personnel handling the linens may not be wearing protective gloves, allowing possible transfer of pathogens from handler to patient. In order to strive for more sanitary conditions, antimicrobial coatings and treatments of textiles can potentially be used to actively fight the

growth of microorganisms after they've been washed. By eliminating pathogens in a patient's direct environment, antimicrobial linen can potentially reduce the risk of infectious disease transfer in the clinical environment.

Titanium dioxide is a semiconductor metal oxide that is used in a variety of coating applications. It is capable of degrading organic matter through photocatalytic activity under certain circumstances. Surface photocatalysis provides very harsh conditions for microbial growth (Abidi, 2009). It was first discovered in the 1960s by Akira Fujishima. He observed the formation of radicals by photocatalytic breakdown of water in the presence of  $\text{TiO}_2$  on Pt electrodes under exposure to UV radiation (Fujishima, 2006). Photocatalysis of titania occurs when light is absorbed by a  $\text{TiO}_2$  particle. The particle then creates electron holes which will generate hydroxyl radicals. Therefore,  $\text{O}_2$  and  $\text{H}_2\text{O}$  can be reduced to form peroxides. The instability of most hydroxyl radicals and peroxide molecules allows them to decompose organic molecules on contact (Abidi, 2009). The reactivity of a photocatalytic surface serves a variety of applications. For example, immobilized  $\text{TiO}_2$  nanoparticles have been commonly used as coatings for indoor odor removal, antimicrobial surfaces, self-cleaning surfaces, and water treatment. (Fujishima, 2006).  $\text{TiO}_2$  containing wall paper and air fresheners are used because they decompose ammonia, hydrogen sulfide, acetaldehyde, toluene, methyl mercaptan, and nitrogen in the nearby area (Fujishima, 2006). In previous studies of titania coated wall tiles, glass plates, and linens, photocatalytic  $\text{TiO}_2$  killed *E. coli* and even decomposed its dead cells (6). It has been reported that nanoparticle titania is utilized as exterior architectural coatings and has proved itself to be useful for its ability to decompose octadecane, glycerol, triolate, and PEG under very weak UV illumination while exhibiting self-regeneration (Fujishima, 2006). This application has the ability to destroy organic pollutants or soil buildup on many exterior surfaces



(Fujishama, 2006). Photocatalytic titania has been reported for its ability to treat toxic chemicals in British river water without creating any toxic byproducts (Fujishama, 2006). In addition, its use as a super-hydrophilic agent has been successfully used for anti-fogging films and self-cleaning glass (Fujishama, 2006). Photocatalytic  $\text{TiO}_2$  is highly applicable due to its price, chemical stability, and properties (Fernando, 2009).

In this study, a titania coating was synthesized in the form of a nanosol, and coated onto hospital linen. The nanosol is a nanoparticle dispersion that consists of an inorganic metal oxide dispersed in an organic matrix. This is often made feasible by the use of sol gel chemistry. A sol-gel process is carried out through the combination of a low molecular weight organic precursor and an inorganic precursor such as an inorganic alkoxide (Fernando, 2009). There are many uses for titania nanosols. It has been reported that a sol gel process using a titanium alkoxide with ethanol in acidic conditions has been successful in creating an anti-microbial and self-cleaning coating on textiles (Wu, 2009; Daoud, 2005). Titania nanosols have been reported to have the ability to be photo-activated which induces antimicrobial activity once coated onto textiles. (Rahal, 2011; Dastjerdi, 2010). The efficiency of a titania nanosol surface treatment was characterized with scanning electron microscopy (SEM), and antimicrobial resistance is measured by both Kirby-Bauer diffusion-type assays (KBA), and Post-antibiotic Effect (PAE), both of which are common for determining an antibiotics efficacy (Braga, 2004; Drew, 1972).

## **Experimental**

### Nanosol Preparation:

Titania nanosol was synthesized using 10 mL of titanium (IV) butoxide precursor purchased from Sigma Aldrich. This inorganic alkoxide was dissolved in 25 mL of ethanol and added drop wise to 200 mL of a .04 M nitric acid solution in a 500 mL round bottom flask while being stirred at room temperature for 48 hours to ensure that the hydrolysis reaction takes place to form titania crystals. This nanosol was set to stand at room temperature for an additional 72 hours before it could be used for the coating application. The TiO<sub>2</sub> content was measured using a TA Instruments Q-500 Thermogravimetric Analyzer (TGA). The dispersion was measured to contain 0.66% solids.

### Coating Application:

The titania nanosol was coated onto a freshly cleaned bed sheet using a dip coating technique. A brand new hospital bed sheet, T-180 thread count, 65 % cotton, 35 % nylon, was used. The bed sheet was sterilized by boiling for 10 minutes, then washed at 60°C with 50 mL Tide<sup>®</sup> Original Scent liquid laundry detergent and 100 mL of deionized water using a 500 mL beaker for 30 minutes to detach any dirt or impurities that might inhibit the coating from adhering to the fabric. The fabric was then dipped in ethanol, washed with water to remove any excess detergent, and dried overnight in a 60 °C oven. The bulk fabric was cut into 1 in<sup>2</sup> pieces. Three pieces were set aside and three were submersed in a titania nanosol for 2.5 minutes with mixing. It was pressed between two pieces of filter paper to remove excess liquid and then placed in a 60 °C oven for five minutes to cure the coating. Oven temperature must be increased to 115 °C to facilitate attachment of titania to fiber as reported by Wu et al. (Wu, 2009) which increases the formation of titanium dioxide crystals. Coated textiles were placed in a hot water

bath at 70 °C for 2 hours to remove any unbound titania particles from the textile to leave a cleanly coated surface.

#### Surface Characterization:

Textile surfaces were characterized via SEM for adhesion of titania particulates. Linen samples (coated and uncoated) were cut to 0.25 in<sup>2</sup> pieces. In order to increase electrical conductivity of linen, it was sputtered with a 50 Å thick layer of gold which increased the focusing potential. Specimens were examined at approximately 500, 2000, and 10,000X magnification. Energy Dispersive X-ray analysis (EDX) was carried out at the low and high magnifications (500/10,000X) to determine the elemental composition of both coated and non-coated substrates. Areas which were found to have high content of titania by EDX were characterized.

#### Antimicrobial Activity

Antimicrobial activity was analyzed using two separate assays; KBA and PAE. A KBA is used to determine the resistance of a bacterial or viral strain to a certain antibiotic or drug (DeCross, 1993). Typically, a cotton disk is saturated in an antibiotic or detergent and is placed on a growth medium such as tryptic-soy agar (TSA) containing a freshly coated liquid layer of a diluted bacterial colony. The dish is then incubated at 35°C for 24 hours (temperature most suitable for *S. Aureus*) and the “zone of inhibition” is measured. The area surrounding the disk where no colonies have formed indicates the “zone of inhibition”. The diameter of this circular area is significant of a bacteria’s resistance to a specific antibiotic. This diameter is strongly dependent on the concentration of antibiotic, bacterial species and temperature of incubation (Drew, 1972). In this study, 1 in<sup>2</sup> titania coated fabric was tested using KBA. In addition, a PAE is typically used to observe effectiveness of an antibiotic. PAE is measured by the recovery time

of a bacterial colony after it has been exposed to an antibiotic for a given amount of time. The PAE is dependent on the exposure time, bacterial species, and concentration of the antibiotic (Sharma, 2002). The PAE can be defined as the time it takes for a bacterial colony to recover to full growing potential after it has been exposed to an antibiotic and can be measured using absorption spectroscopy. By using absorption spectroscopy, the concentration of microorganism or colony forming units (CFU) can be determined. The PAE can be calculated at the time a bacterial colony has increased to its maximum growth potential or when the absorption on the spectrometer increased by 1 OD<sub>600</sub>. An absorption increase of 1OD<sub>600</sub> indicates an increase of 1 log<sub>10</sub> colony forming units. As bacteria increased by 1 log CFU unit, it indicates that a bacterial colony has returned to its maximum growth rate.

White textiles (same as above) were all tested for their ability to inhibit growth of *S. aureus* on tryptic-soy agar (TSA) agar. *S. aureus* (ATCC 6538) was obtained from the Cal Poly microbiology stockroom and analyzed for purity. It was streaked on a sheep blood agar plate and incubated for 24 hours at approximately 35°C to detect purity. *S. aureus* is a beta-hemolytic species that turns red agar yellow. Stock *S. aureus* was prepared by picking 4-5 typical colonies with a sterile disposable 10 µL loop and re-suspending colony in 25 mL of tryptic-soy broth (TSB) in a sterile Erlenmeyer flask. The flask was placed in a shaker-incubator for 2 hours at 35 °C and then adjusted to .09-.12 D.U. on a Spectronic 20 OD<sub>600</sub> spectrometer which corresponds to about 1x10<sup>8</sup> CFU *S. aureus*. This inoculum was then used for PAE and KBA.

In preparation for the PAE assay, 1 mL of TSB was added to 5 separate test tubes. A 1 in<sup>2</sup> piece of titania-coated white cloth was added to tube 1 and a non-coated piece of cloth was added to tube 2. 1 mL of 25 ppb silver nitrate is added to tube 3. The inoculum described above was diluted 1:10 with pre-warmed TSA and 1 mL was added to tubes 1-4. Tube 4 serves as the

inoculum control (only *S. aureus* and growth medium without fabric) and tube 5 serves as medium sterility containing only the growth medium, (Table 1). The tubes were incubated for 1 hour and the inoculum was plated on TSA using a spread-plate method by pipetting 0.1mL aliquots of  $10^{-5}$  and  $10^{-6}$  dilutions of inoculum to determine viable bacterial count. Tubes 1-6 are incubated for 1 hour and then diluted 1:1000 with pre-warmed TSB and 250  $\mu$ L aliquots were pipetted into sterile micro well plates in duplicate. Growth curves were constructed taking absorbance readings at 600 nm every 10 minutes for 12 hours on a Molecular Devices Spectra Max Plus 384 micro plate reader.

Table 1: Contents of Tubes

<i>Tube #</i>	<i>Contents</i>
1	TiO <sub>2</sub> Coated
2	Uncoated Fabric
3	25 $\mu$ g/L AgNO <sub>3</sub>
4	Inoculum Control
5	Only Growth Medium

A slightly altered KBA method was used. Coated and un-coated textiles were used and observed for growth around the edges as opposed to measuring zone of inhibition surrounding the traditional cotton disk. The textile samples were dried and cured so that the titania nanoparticles could form on the surface of the material. *S. aureus* solution ( $1 \times 10^8$  CFU) was streaked uniformly onto TSA. Six 1 in<sup>2</sup> squares of cloth (3 titania coated, 3 uncoated placed side by side) were placed on fresh bed of *S. aureus* (Figure 1). The plates were placed in an incubator at 35 °C for 24 hours, and then placed on a laboratory bench for 24 hours with illumination from indoor fluorescent light bulbs to encourage photo catalytic activity.

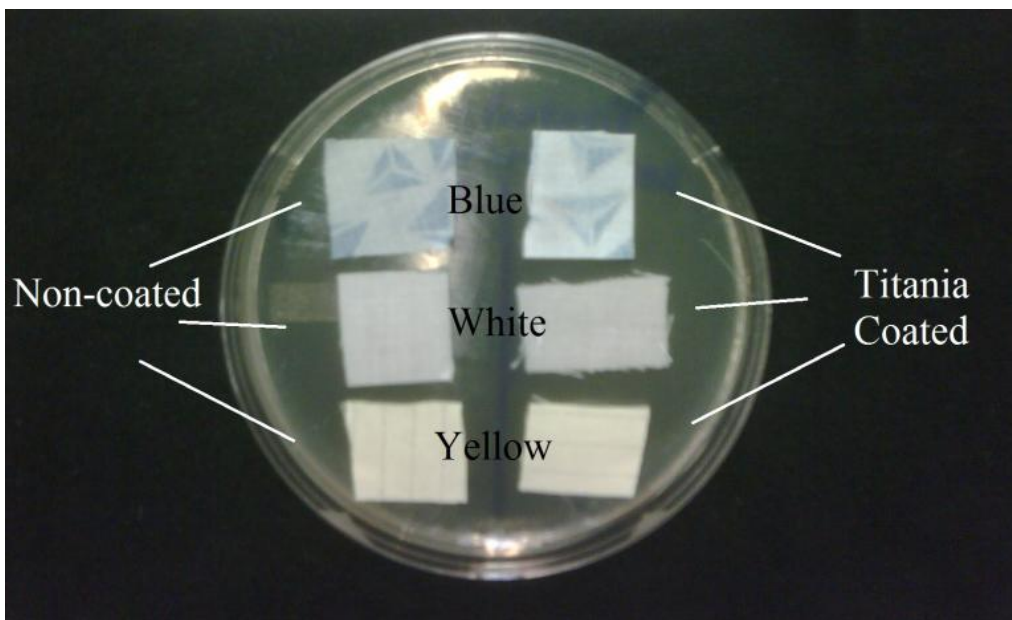


Figure 1: Diagram exhibits the orientation of titania coated vs. non-coated textiles on a bed of *S. aureus*.

## **Results and Discussion:**

### Surface Characterization

Figures 2 and 3 display the SEM images of a coated vs. uncoated linen at 500 X magnification. It is difficult to distinguish individual fibers in Figure 3 compared to easily distinguished fibers in Figure 2. The difference in clarity is most likely be a result of the added titanium dioxide particles. Figure 2 also exhibits a much greater depth of field than Figure 3. This may indicate that there is less free space due to the presence of titania. This tightly packed network makes it difficult to perceive depth in the image. It was also observed that the fabrics were physically stiffer and brittle when coated compared to a pristine (uncoated) fabric. At low magnifications, the EDS exhibited significant peaks for carbon and oxygen as expected. Carbon and oxygen are the key elements of the polymer chains that make up cotton and nylon fibers. The EDX displayed trace amounts of gold due to gold sputtering procedure used to enhance electrical conductivity for SEM analysis. Titania coated fabric exhibited a large signal of titanium at 15.05 % weight as determined by EDX software.

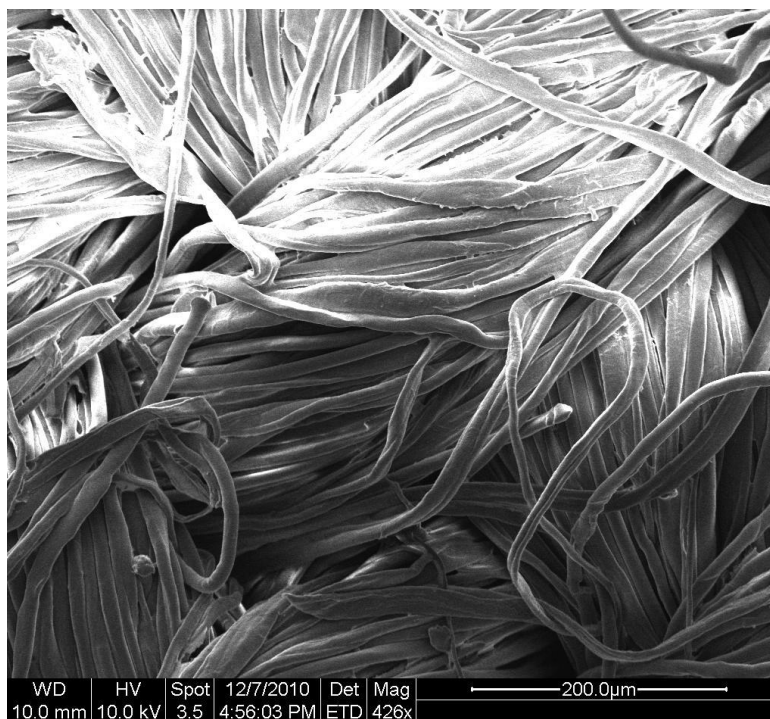


Figure 2: SEM Image of uncoated white fabric at 500 X magnification.

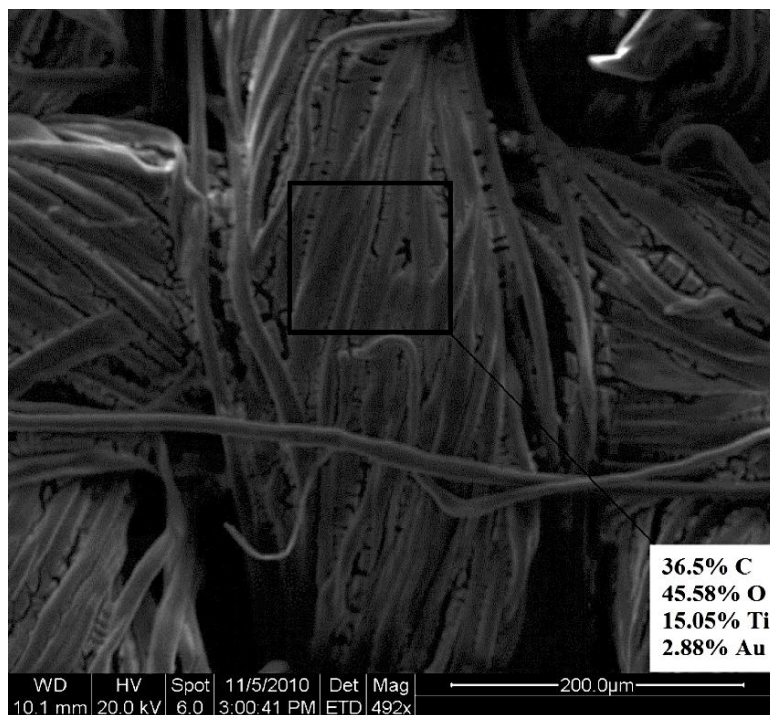


Figure 3: SEM image of titania coated white fabric at 500 X magnification.



At higher magnifications (greater than 1500 X) particle formations are observed between the fibers (Figure 5) rather than on the surface of the fibers. At approximately 2000 X magnification there is little difference in depth of field between coated (Figure 5) and uncoated (pristine) fabrics (Figure 4). The coating formed a branching network throughout the specimen in Figure 5, while the non-coated fabric displayed very smooth surfaces on the fibers with free space in between them.

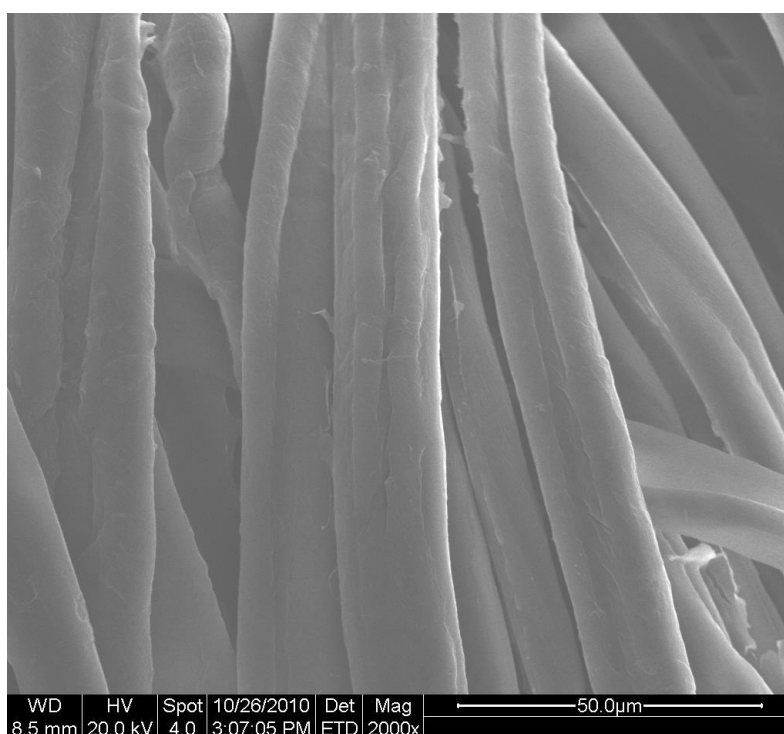


Figure 4: SEM image of uncoated white fabric at 2000 X magnification.

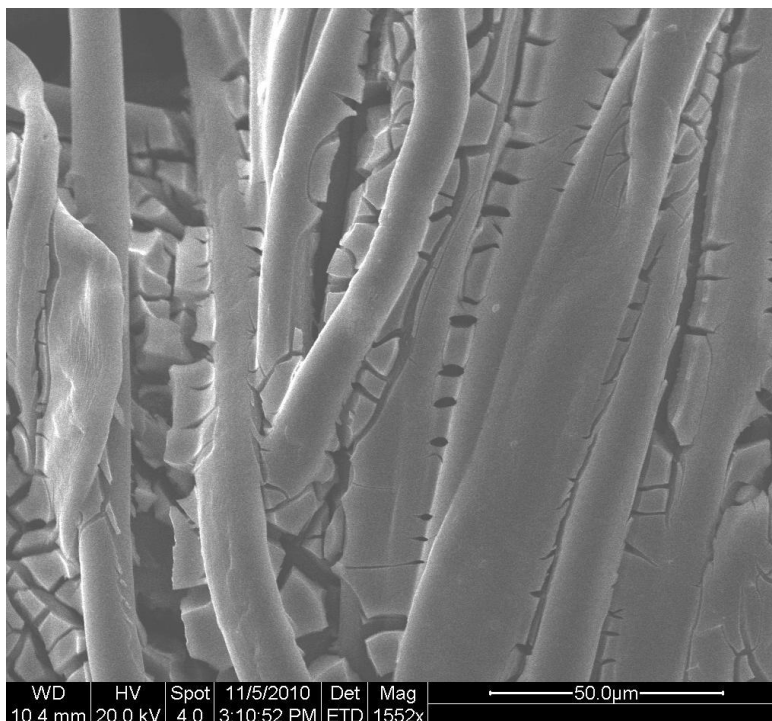


Figure 5: SEM image of titania coated white fabric at 1500 X magnification.

Figures 6 and 7 display the SEM images of very high magnifications of coated and pristine fabrics. Unfortunately, the SEM could not focus above 8000 X, before losing focus, but was still comparable with the high magnification of the non-coated fabric in Figure 6 which allowed focus as high as 12000 X. At this magnification, the non-coated fabric shows a very clear depth of field, while the coated fabrics are very flat in their image. Titania formed interstitially between various fibers of coated fabric (Figure 7). Titania particle sizes were consistently between 5 and 10 μm. EDS characterized them to be titanium and oxygen indicating that they are indeed titanium dioxide microparticles rather than nanoparticles. EDS also displays the placement of titania particles in the fiber matrix. It is clear that the composition of the interstitial area is largely made up by titanium and oxygen (32 and 46 % weight), which is likely

to be titanium dioxide (Figure 7). The fiber surfaces itself contains very little titanium (2.21 % weight) compared to the interstitial space between fibers. The network of titania particles may contribute to the observed stiffness of this textile.

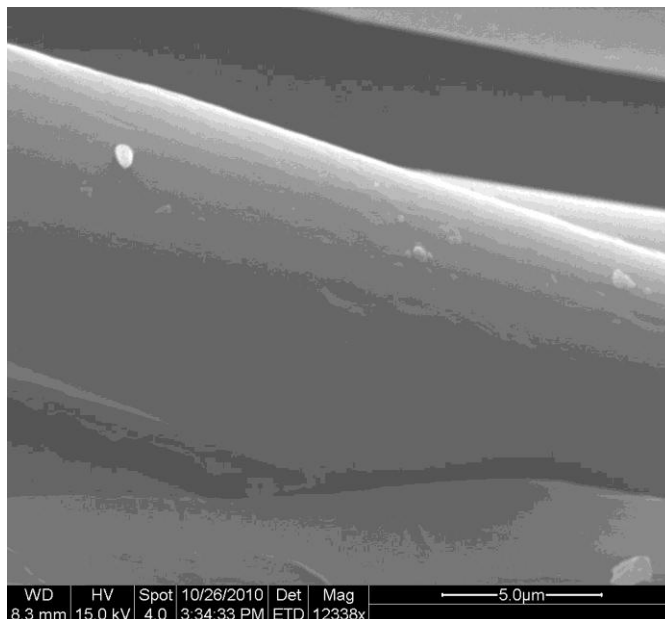


Figure 6: SEM of Uncoated white fabric at 12000 X.

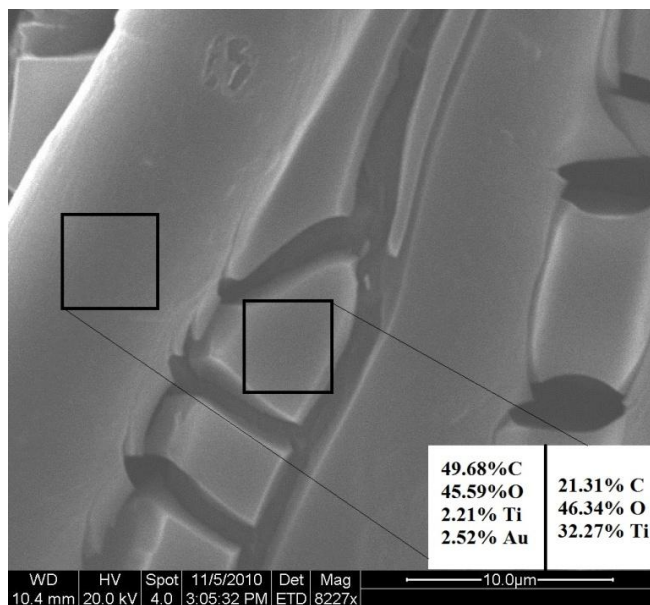


Figure 7: SEM Titania coated white fabric at 8000 X.

### PAE of Titania Coated Fabrics

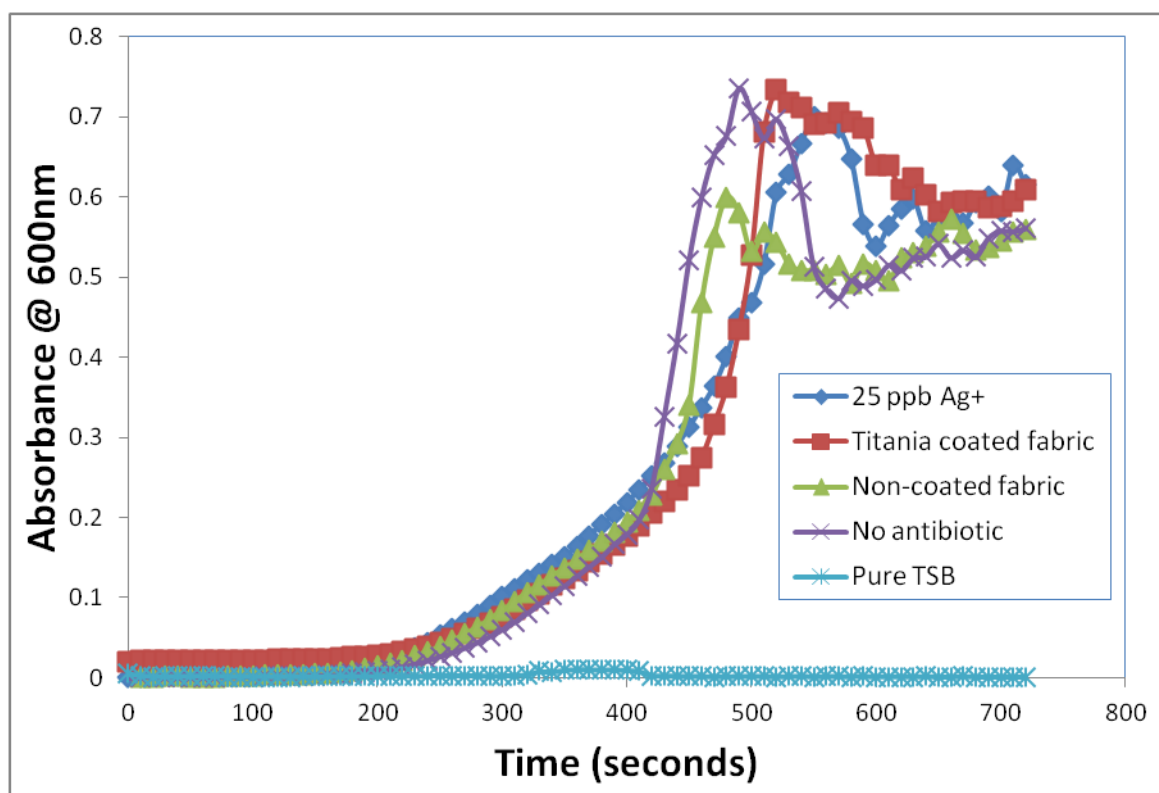


Figure 8: PAE on *S. aureus* of titania coated fabric, AgNO<sub>3</sub>, pristine fabric, and no other things in TSB.

Table 2: PAE results of titania coated textile versus silver.

Antibiotic	PAE (hours)
Titania coated fabric	1
Pristine fabric	0.2
25 ppb AgNO <sub>3</sub>	1.2

The PAE of a titania coated films is exhibited in Figure 8. The absorbance values correlate to CFUs of *S. aureus* forming after the antibiotics (titania and silver nitrate) have been removed. As soon as the curve reaches its steepest point, *S. aureus* has reached its maximum growth rate, meaning that the antimicrobial activity has diminished. A trace amount, 25 parts per billion (ppb), of silver nitrate was used because of its known antimicrobial activity in aqueous solutions (Roy, 2007) which were used for a standard antibiotic on the PAE. Although a small

amount is used, AgNO<sub>3</sub> was able to create a PAE of 1.2 hours in an ultra-dilute solution.

Considering that these antibiotics were only exposed to a *S. aureus* for 1 hour, a 1 hour lasting effect is substantial compared to other antibiotics (Sharma, 2002). Titania coated textiles were also able to suppress the bacterial growth by resulting in a PAE of 1 hour. The uncoated fabric shows an inhibition of growth for 0.2 hours. This inhibition may be due to the fact that the substrate is not the most applicable growth medium for *S. aureus* making it difficult for bacteria to grow. It is possible that the 1 hour PAE observed in the titania coated samples is due to photocatalytic antimicrobial activity. Typical PAEs may last between 1 and 3 hours but will not completely describe the antimicrobial activity of a compound (Braga, 2004).

#### Kirby Bauer Assays

KBAs determine antimicrobial efficacy and may suggest photo catalytic activity of the TiO<sub>2</sub> coated fabric. The KBA is shown for six different fabrics. Three were coated with titania (Figures 9d, 9e and 9f) while three were uncoated (9a, 9b, and 9c). Figure 9 displays the inoculated medium TSA plate after textiles have been removed. From top to bottom was blue nylon/cotton blended textile (9a and 9d), white nylon/cotton blend (9b and 9e), and yellow carbon laced nylon textile (9c and 9f). This was done to measure the visual regrowth around the edges of the cloth. All of the titania coated fabrics exhibited a low level of bacterial growth around their edges (Figures 9d,9e, and 9f). Both untreated blue and white textiles encouraged bacterial growth, but yellow did not. Unlike cotton/nylon blended fabrics, the yellow fabric was composed of a mixture of nylon fibers laced with carbon. It is clear that *S. aureus* was unable to grow around the edges and underneath this fabric sample whether or not it was coated in titania. The activated carbon may contribute to antimicrobial behavior of uncoated textile. Overall, the lack of growth around the white and blue coated fabrics compared to the non-coated fabrics

indicate that there is possible antimicrobial activity occurring. The plates may be exhibiting photocatalytic activity due to indoor laboratory luminescence. The radical formation would therefore inhibit microbial growth around the edges of the cloth.

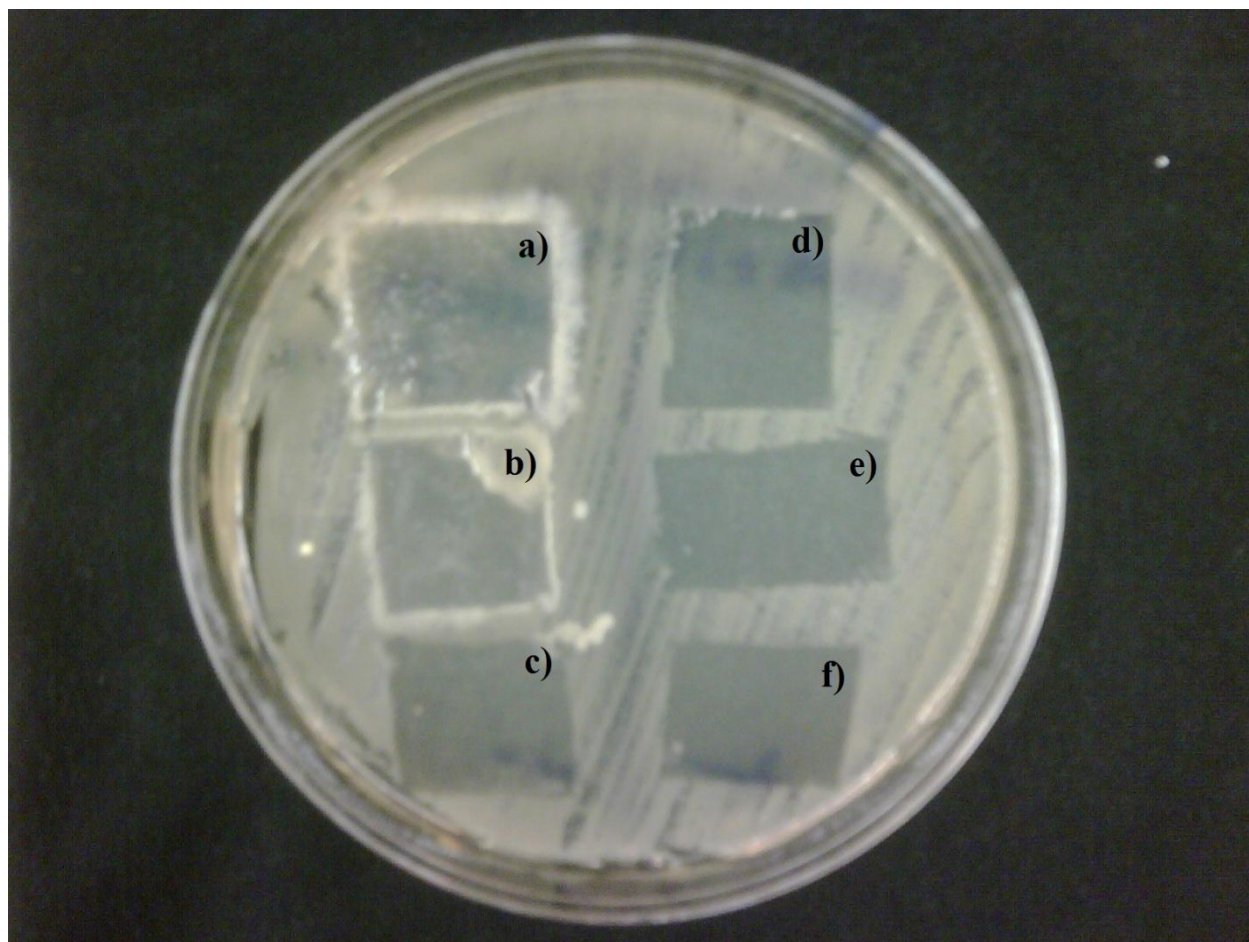


Figure 9. Titania coated fabric areas are displayed on the right while pristine fabric areas are displayed on the left side of the Petri dish. a) and d) are blue cotton/nylon blended fabric, b) and e) are white cotton/nylon blended fabric, and c) and f) were carbon laced nylon.

## Conclusion

A Titanium dioxide nanosol was prepared from a hydrolysis reaction of titanium (IV) butoxide and ethanol. This nanosol was used as a coating for cotton and nylon blended fibers and subjected to a series of tests. SEM characterization displayed formation of titania particle formation in the interstitial spaces between fibers. The particles were determined to contain mostly titanium and oxygen by EDS analysis, confirming that the particles were most likely pure titanium dioxide particles. SEM instrument was unable to focus at high magnifications due to lack of electrical conductivity. Although a gold sputtering technique was used to increase electrical conductivity of sample, magnifications on the nano-scale were not obtained. Although larger than expected in size, SEM showed successful adhesion of titania particles in between threads of nylon/cotton textiles.

In biological analysis, titania coated textiles inhibited growth of *S. aureus* on and around its surface, and produced post- antibiotic effects on *S. aureus* in TSB. The prevention of future growth was exhibited in a PAE of 1 hour, meaning that it took *S. aureus* 1 hour to recover and resume its maximum growth rate once the titania coated fabric was removed from its growth medium. KBA displayed an inhibition of growth around the edges of titania coated textile, but not around pristine substrates. In fact, *S. aureus* colonized favorably around pristine fabric on a TSA growth medium. It's possible that textile samples were not completely sterilized in the pre-treatment process. Linens were pre-treated by boiling in water for 10 minutes. It's possible that the textile samples were contaminated with endosporic *bacillus* (able to withstand very high temperatures and pressures) prior to the boiling process. It might be that excess growth around the edges of the untreated nylon/cotton textile in KBA is just contamination from earlier contact. In future experiments, it would be more aseptic to autoclave the textile samples for completely

sterile testing subjects to ensure that all spore forming organisms are killed. In addition to further microbiological tests, future work might include the study of adhesion efficiency of titania particles and antibacterial activity dependence on particle concentration. In addition to experimental improvements, the health effect of a titania coated textile for human application could be studied in future work. Before an application like this can be used in a clinical setting, its health effects must be studied. Photocatalysis and its effect on the skin (for use of titania textiles in clothing) needs to be investigated.

In conclusion, titanium (IV) butoxide was successfully used to synthesize a coating on the cotton/nylon substrates. The coating exhibited possible antimicrobial ability, but further research must be conducted to determine existence of photocatalytic activity. Because of titania's cost and ease of use, it has potential in the field of antimicrobial coatings to reduce the spread of infectious disease on surfaces. Although linen is not commonly known as a bacterial vector, it is gaining much more attention in hospitals and other clinical settings as an infectious disease carrier. Titanium dioxide's ability to photo-degrade small compounds has already been utilized, but it has far greater potential and may contribute to saving lives by reducing the risk of infectious disease.



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