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Atomic Layer Deposition of Titanium Oxide Thin Films on Nanoporous Alumina Templates for Medical Applications

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Abstract

Nanostructured materials may play a significant role in controlled release of pharmacologic agents for treatment of cancer. Many nanoporous polymer materials are inadequate for use in drug delivery. Nanoporous alumina provides several advantages over other materials for use in controlled drug delivery and other medical applications. Atomic layer deposition was used to coat all the surfaces of the nanoporous alumina membrane in order to reduce the pore size in a controlled manner. Both the 20 nm and 100 nm titanium oxide-coated nanoporous alumina membranes did not exhibit statistically lower viability compared to the uncoated nanoporous alumina membrane control materials. In addition, 20 nm pore size titanium oxide-coated nanoporous alumina membranes exposed to ultraviolet light demonstrated activity against *Escherichia coli* and *Staphylococcus aureus* bacteria. Nanostructured materials prepared using atomic layer deposition may be useful for delivering a pharmacologic agent at a precise rate to a specific location in the body. These materials may serve as the basis for "smart" drug delivery devices, orthopedic implants, or self-sterilizing medical devices.

Manuscript Body

Recent industrial and academic research efforts have focused on the development of nanostructured materials for use in biomedical devices, including medical prostheses, implantable biosensors, and drug delivery devices. Nanostructured materials are defined as materials that contain clusters, crystallites, molecules, or other structural elements with dimensions in the 1 nm-100 nm range.[1] Recent advances in the use of nanostructured materials for medical applications have resulted from two motivations. First, there is a natural evolution to nanoscale materials as novel processing, characterization, and modeling techniques become available. Second, specific interactions between biological structures (e.g., enzymes and other proteins) and nanostructured materials may allow for devices with unusual functionalities to be developed.

For example, nanostructured materials may play a significant role in controlled release of pharmacologic agents for treatment of cancer.[2] Systemic administration (distribution throughout the entire body) of many common chemotherapeutic agents is associated with significant side effects. For example, the side effects of the common chemotherapeutic agent doxorubicin hydrochloride include myelodysplasic syndrome, congestive heart failure, and mucositis. In addition, many protein- and DNA- based treatments that are being developed for treatment of cancer have relatively short in vivo activities. These chemotherapeutic agents cannot be administered in oral form, because they may be metabolized by the liver, intestine, kidneys, or lungs before reaching systemic circulation. Recent work has examined delivery of chemotherapeutic agents at the site where they are needed; this route avoids diffusional and enzymatic barriers and provides complete and instantaneous absorption. Nanostructured materials may provide constant delivery of a pharmacologic agent to the site in the body where it is needed, providing appropriate treatment over an extended time while minimizing damage to healthy tissue.

Nanoporous polymer materials are inadequate for use in drug delivery. Many porous polymer materials are created using solvent-casting techniques.[3] These materials have poor mechanical properties and large pore size distributions; for example, pore size variation is as large as 30%. In addition, polymer membranes contain 100–200 μm tortuous pores. Ion-track etching has also been used to form membranes. This technique produces a much narrower pore size distribution than that observed in polymer membranes; for example, pore variation in membranes produced using ion-track etching is within 10%. However, ion-track etched membranes have low

porosities; pore concentrations under 10^9 pores/cm^2 are commonly observed. Porous silicon is another material that has been considered for use in drug delivery.[4,5] This material may be produced by electrochemically corroding silicon in solutions containing hydrofluoric (HF) acid. The pores propagate in the <100> direction of silicon. The average diameter of the nanocrystalline porous silicon layers can be modified by altering the electrolyte composition, the electrochemical current, or the dopant characteristics. It should be noted that porous silicon films undergo degradation under physiologic conditions. Although several investigators have examined the use of porous silicon for drug delivery, it is unclear whether how patient-to-patient differences in physiologic status may affect degradation of porous silicon or drug release rates.[6]

Nanoporous alumina provides several advantages over other materials for use in controlled drug delivery and other medical applications. Matsuda et al. demonstrated that anodization, stripping of the oxide, and re-anodization produces an unusual material with nanoscale pores.[7] Nanoscale pores are randomly formed on the alumina surface at the beginning of the anodization process. These pores self-organize into a hexagonal arrangement during their growth into the bulk material. This first oxide layer is removed using an aqueous solution of 1.8 wt% Cr (VI) oxide and 6 wt% phosphoric acid. A second anodization process is carried out on this template. The resulting material, known as “alumite”, contains long, columnar, ordered nanopores. These nanopores demonstrate long range order. The structure can be described as close-packed cells in a hexagonal arrangement, with pores at the center of each cell. The pore size can be modified by selection of appropriate processing temperature, electrical field strength, or electrolyte.

Nanoporous alumina provides several advantages over polymers for use in drug delivery. Alumina is a bioinert ceramic that is stable in physiologic solutions. Nanoporous alumina membranes can be processed with smaller pore sizes (20–100 nm range) and more uniform pore sizes than polymer membranes. Finally, the anodization process provides precise control over pore size and pore distribution. However, there is a significant disadvantage to the use of nanoporous alumina materials in medical applications. Although aluminum is a constituent of several medical alloys (e.g., Ti-6Al-4V, ASTM F136), it is currently unknown whether aluminum is a biocompatible material.[8] In this paper, a nanoporous alumina membrane was coated with titanium oxide using atomic layer deposition.[9] The biocompatibility and corrosion resistance of titanium oxide is well known.[10] In fact, titanium oxide is commonly used as a passivation layer in dental, orthopedic, and cardiovascular implants.[11-13] Having a conformal coating of titanium oxide over the alumina membrane is of importance in order to minimize corrosion and improve cell compatibility. Previous work by Canabarro et al. has indicated that cell grown on titanium oxide surfaces contaminated by small amounts of alumina exhibit impaired activity; for example, contamination by alumina may lead to impaired mineralization of matrix by osteoblasts (bone cells).[14]

Atomic layer deposition was used to coat all the surfaces of the nanoporous alumina membrane in order to reduce the pore size in a controlled manner.[9] Recent work by Kipke et al. on unmodified nanoporous alumina membranes has shown that release of biological molecules in nanoporous alumina membranes is determined by the diameters of the pores.[15] The titanium oxide coating will reduce the pore size in a controlled manner, maintain a narrow pore size distribution, prevent aluminum ions from leaching into the surrounding tissues, and create a biocompatible pore/tissue interface.

Atomic layer deposition (also known as molecular layering or atomic layer epitaxy) is a thin film growth technique in which alternating chemical reactions occur between gaseous precursor molecules on a surface.[16, 17] The self-terminating gas-solid reactions allow for material to be

deposited in a layer-by-layer fashion. Individual reactions are separated by purge steps that involve saturation with an inert gas. By saturating the substrate at each individual reaction, all surfaces of a given substrate can receive a conformal coating of identical thickness. The atomic layer deposition cycles can be repeated to control precisely the coating thickness.

The self-limiting nature of the reaction between these precursors and the surface ensure that all exposed regions of a substrate, including areas that are only accessible via long, tortuous pathways, are coated uniformly and precisely. It is this ability to produce conformal coatings on non-planar substrates makes atomic layer deposition very useful for functionalizing nanoporous materials, including membranes and aerogels. The conformal capability of atomic layer deposition is quite different from that of physical vapor deposition technologies such as evaporation and sputtering, which are limited by line-of-site constraints and can only coat the outer surface of a porous substrate. As such, atomic layer deposition is uniquely suited for depositing a conformal nanometer-scale film with precise thickness onto the surface of a nanoporous membrane.

Atomic layer deposition technology has been developed over the last thirty years. This technology has been commercialized for a range of applications in microelectronics, including electroluminescent displays, logic chips, and disk drives. There is currently intense interest in developing atomic layer deposition methods to a range of new applications outside of the realm of microelectronics. Much of the ongoing work involves coating nanoporous or nanostructured templates to impart the templates with beneficial chemical or physical functionalities. For example, nanoporous alumina membranes are a convenient platform for synthesizing nanotubes and nanowires using atomic layer deposition-based templating methods.

Following the atomic layer deposition of the titanium oxide coating, ellipsometry of the Si(100) witness samples yielded a coating thickness in the range of 7.6-9.2 nm. These thicknesses yield an atomic layer deposition growth rate for titanium oxide of 0.86-1.0 Å/cycle, which is in agreement with previous reports. Scanning electron microscopy was performed on titanium oxide-coated nanoporous alumina membranes using a S4700 microscope (Hitachi, Tokyo, Japan) with field emission gun electron beam source. Scanning electron micrographs obtained from the large-pore side of the nanoporous alumina membranes (Fig. 1a) show the openings of the pores with diameters of ~200 nm. A higher resolution scanning electron micrograph of the nanoporous alumina membrane surface (Fig. 1b) reveal nanocrystals with lateral dimensions of ~20 nm. These nanocrystals were not observed on the uncoated nanoporous alumina membranes (SEM images not shown) and therefore are consistent with titanium oxide nanocrystals from the atomic layer deposition coating. In addition, cross-sectional scanning electron micrographs obtained after fracturing the membranes demonstrate that the titanium oxide nanocrystals extend to the middle of the 60 micron nanopores (Fig. 1c). Raman spectroscopy was performed on the nanoporous alumina membranes before and after the atomic layer deposition of titanium oxide coatings using a RM2000 Raman microprobe spectrometer (Renishaw, Hoffman Estates, Illinois). Prior to the titanium oxide coating, the Raman spectrum is featureless. After atomic layer deposition of titanium oxide coating, the Raman spectrum show distinct bands corresponding to the anatase TiO_2 peaks (Fig. 2). In addition, powder X-ray diffraction measurements performed on the TiO_2 -coated nanoporous alumina membranes (not shown) also confirm the anatase phase.

Annealed titanium oxide-coated nanoporous alumina membranes and uncoated nanoporous alumina membranes with 20 and 100 nm pore size were sterilized using ultraviolet light. All membranes were exposed for three hours on each side and rotated 90° every forty-five minutes

to ensure that all sides were sterilized. Upon completion of ultraviolet sterilization, the membranes were placed in twenty-four well plates using Akwa Tears® (Akorn, Buffalo Grove, Illinois) to prevent floating of the membranes. Human epidermal keratinocytes (HEK) were seeded with 20,000 HEK in 1 ml of KGM-2 per well in twenty-four well plates. Media was changed after twenty-four hrs. Once human epidermal keratinocytes were 60% confluent (24 hrs after seeding) timed sampling began. Media was then harvested after 24 hrs and stored at -80°C and the Human epidermal keratinocytes were assayed for viability by MTT (3-[4,5-dimethyl-2-thiazol]-2,5-diphenyl-2H-tetrazolium bromide).[18] The membranes were moved to a new twenty-four well plate so Human epidermal keratinocytes grown in the wells did not influence the data. Human epidermal keratinocytes were incubated under cell culture conditions in MTT medium (0.5mg MTT per 1 ml KGM-2) for three hours. The cells were rinsed in Hank's Balanced Salt Solution and the tetrazolium metabolized in the mitochondria extracted with 70% isopropanol. The absorbance was quantitated at λ=550nm in a Multiskan RC plate reader (Labsystems, Helsinki, Finland). The data were normalized to the uncoated nanoporous alumina membrane and were expressed as percent viability. Each coating/treatment was run in triplicate. Human epidermal keratinocyte viability was statistically compared using ANOVA (SAS 9.1 for Windows, Cary, NC). Comparisons were made between uncoated and coated membranes using the Student's *t*-test at $p<0.05$. Both the 20 nm and 100 nm titanium oxide-coated nanoporous alumina membranes did not exhibit statistically lower viability compared to the uncoated nanoporous alumina membrane controls. It should be noted that the increase in viability for the titanium oxide-coated nanoporous alumina membrane may be explained by the interaction between the titanium oxide and the MTT assay marker; recent work suggests that nanomaterial-dye physical interactions can affect results in dye-based assays.[19,20]

Two bacteria cultures, *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 (American Type Culture Collection, Manassas VA), were used to examine the antimicrobial properties of titanium oxide-coated and uncoated nanoporous alumina membranes. Both bacteria were cultured overnight to log phase in tryptic soy broth (Difco, Detroit, MI) prior to experimental procedures. Cultures were washed three times and the membranes were sterilized. Cell densities were brought to 10^7 cell/ml and examined using a spectrometer before testing. Coated and uncoated membranes were mounted (two each) on sterile microscope slides with autoclaved stainless steel clips. Slides were separately placed in sterile Petri dishes, and were covered with either (a) 20 ml of bacteria in phosphate-buffered saline solution or (b) uninoculated phosphate-buffered saline solution. The Petri dishes were placed on a shaker table and incubated for four hours at 25 °C. The Petri dishes containing titanium oxide-coated nanoporous alumina membranes were exposed to a Woods Light ultraviolet source. After four hours the Petri dishes were removed from the incubator. Bacteria and/or phosphate-buffered saline solution were removed from the Petri dishes using pipettes. The Petri dishes were subsequently washed three times with phosphate-buffered saline solution. Slides were air dried in a sterile hood, stained with Acridine Orange for two minutes, and then washed three times with filter sterilized (2 µm) deionized water. Ten fields were counted on each filter using a Model 510 laser scanning confocal microscope (Zeiss, Thornwood, NY). Microbial results showed a significant decrease in the amount of S. aureus and E. coli attachment to 20 nm pore size titanium oxide-coated nanoporous alumina membranes as compared to their uncoated counterparts (Table 1). On the other hand, the larger (100 nm) pore size titanium oxide-coated nanoporous alumina membranes did not show an antimicrobial effect. Figure 4 shows controls with phosphate-buffered saline solution, demonstrating as few as 1 cell/field. Figure 5

demonstrates *S. aureus* growth on a 20 nm pore size uncoated nanoporous alumina membrane. Small cell aggregates were also observed on the 20 nm pore size uncoated nanoporous alumina membrane (Figure 6). Recent work by Wong et. al. demonstrated that nitrogen-doped titanium oxide has activity against pathogenic microorganisms, including *Staphylococcus aureus, Streptococcus pyogenes,* and other bacteria found in hospital settings.[21] Maness et al. demonstrated that titanium oxide photocatalysis promotes peroxidation of *E. coli* phospholipid membranes, resulting in cell death.[22] These findings could be attributed to lytic activity induced by the ultraviolet light-titanium oxide interaction. It is interesting to note that the 20 nm pore size titanium oxide-coated nanoporous alumina membrane inhibited microbial adhesion while the 100 nm pore size titanium oxide-coated nanoporous alumina membrane did not (Figure 7). Differences in photocatalytic activity or membrane-cell interaction with membrane surface area may account for this finding.

Nanostructured materials prepared using atomic layer deposition may be useful for delivering a pharmacologic agent at a precise rate to a specific location of the body. Ceramic materials are associated with less inflammation than polymeric materials that are currently used for local drug delivery. In addition, nanoporous ceramics may provide greater control over release rate than polymers, since there are often difficulties when pharmacologic agents and polymers are dissolved in a given solvent.[23] These materials may serve as the basis for “smart” drug delivery device, which allows for controlled release of a pharmacologic agent in response to electric field, magnetic field, pH, temperature, or light intensity. A smart system could release a gene or drug at a precise rate to the location in the body where it is needed. Nanoporous titanium coatings may also be useful for improving bone synthesis in orthopedic implants or in preventing infection. [24, 25]

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References

1. O.V. Salata, Journal of Nanobiotechnology, 2 (2004), pp. 3.
2. G. Stylios et al., Injury, 36 (2005), pp. S6-S13.
3. N. Wisniewski et al., Colloids and Surfaces B: Biointerfaces, 18 (2000), pp. 197-219.
4. S.H.C. Anderson et al., Physica Status Solidi A: Applied Research, 197 (2003), pp. 331-335.
5. L.T. Canham, et al., Thin Solid Films, 297 (1997), pp. 304-307.
6. M. P. Stewart et al., Advanced Materials, 12 (2000), pp. 859-869.
7. H. Matsuda et al., Science, 268 (1995), pp. 1466–1468.
8. M.L. Hegde et al., Journal of Molecular Neuroscience. 22: 19-31, 2003.
9. M. A. Cameron et al., Langmuir, 16 (2000), pp. 7435–7444.
10. V. Faust et al., Key Engineering Materials 206 (2002), pp. 1547–1550.
11. A. Canabarro et al., Journal of Biomedical Materials Research, 87A (2008), pp. 588-597.
12. B.D. Ratner, Journal of Biomedical Materials Research, 27 (1993), pp. 837-850.
13. D. Stoeckel et al., European Radiology, 14 (2004), pp. 292-301.
14. A. Canabarro et al., Journal of Biomedical Materials Research, 87A (2008), pp. 588-597.
15. S. Kipke et al., Advanced Functional Materials, 14 (2004), pp. 1184-1188.
16. R. L. Puurunen et al., Journal of Applied Physics, 96 (2004), pp. 7686-7695.
17. H. M. Alsyouri et al., Langmuir, 19 (2003), pp. 7307–7314.
18. T. Mosmann, Journal of Immunological Methods, 65 (1983), pp. 55-63.
19. N. A. Monteiro-Riviere et al., Carbon, 44 (2006), pp. 1070-1078.
20. N. A. Monteiro-Riviere et al., Toxicology and Applied Pharmacology, 234 (2009), pp. 222-235.
20. M. S. Wong et al., Applied and Environmetnal Microbiology, 72 (2006), pp. 6111-6116.
21. P. C. Maness et al., Applied and Environmetnal Microbiology, 65 (1999), pp. 4094-4098.
23. D. Losic et al., Nanotechnology 19 (2008), pp. 24570.
24. Y.Z. Yang et al., Vacuum, 83 (2008), pp. 569-574.
25 S. R. Chae et al., Journal of Membrane Science, 329 (2009), pp. 68-74.

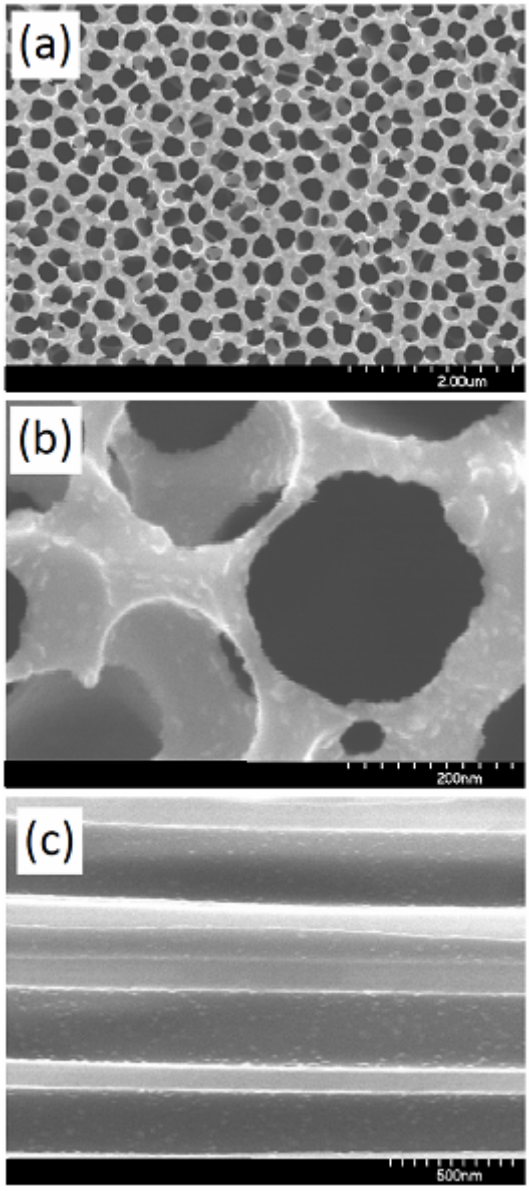

Figure 1: Scanning electron micrographs of a nanoporous alumina membrane following deposition of 8 nm titanium oxide coating using atomic layer deposition.

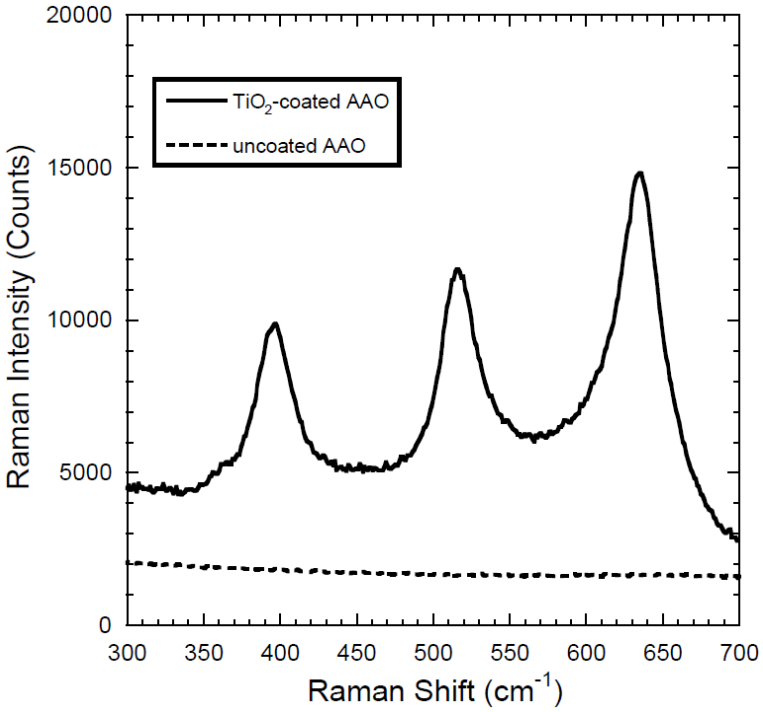

Figure 2: Raman spectra of nanoporous alumina membrane before and after deposition of 8 nm titanium oxide coating using atomic layer deposition, confirming anatase phase of titanium oxide.

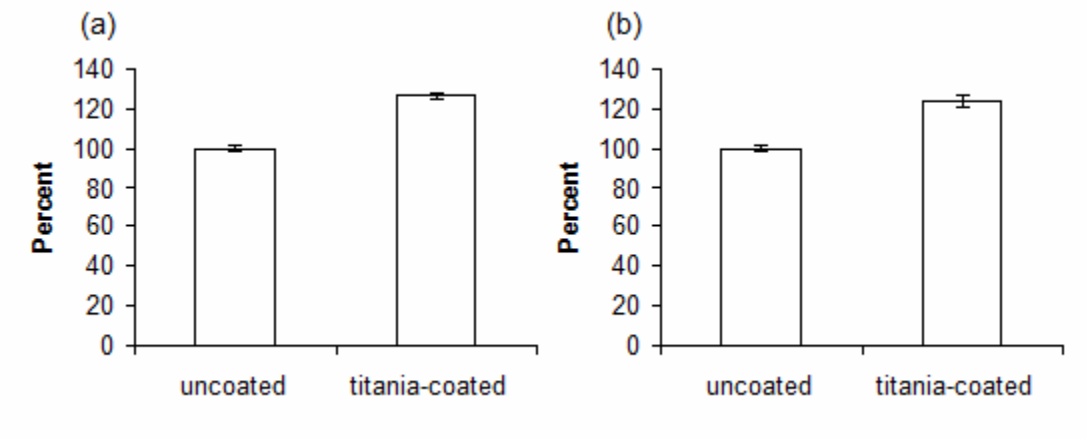

Figure 3. MTT viability of (a) 20 nm pore size and (b) 100 nm pore size titanium oxide-coated and uncoated nanoporous alumina membranes.

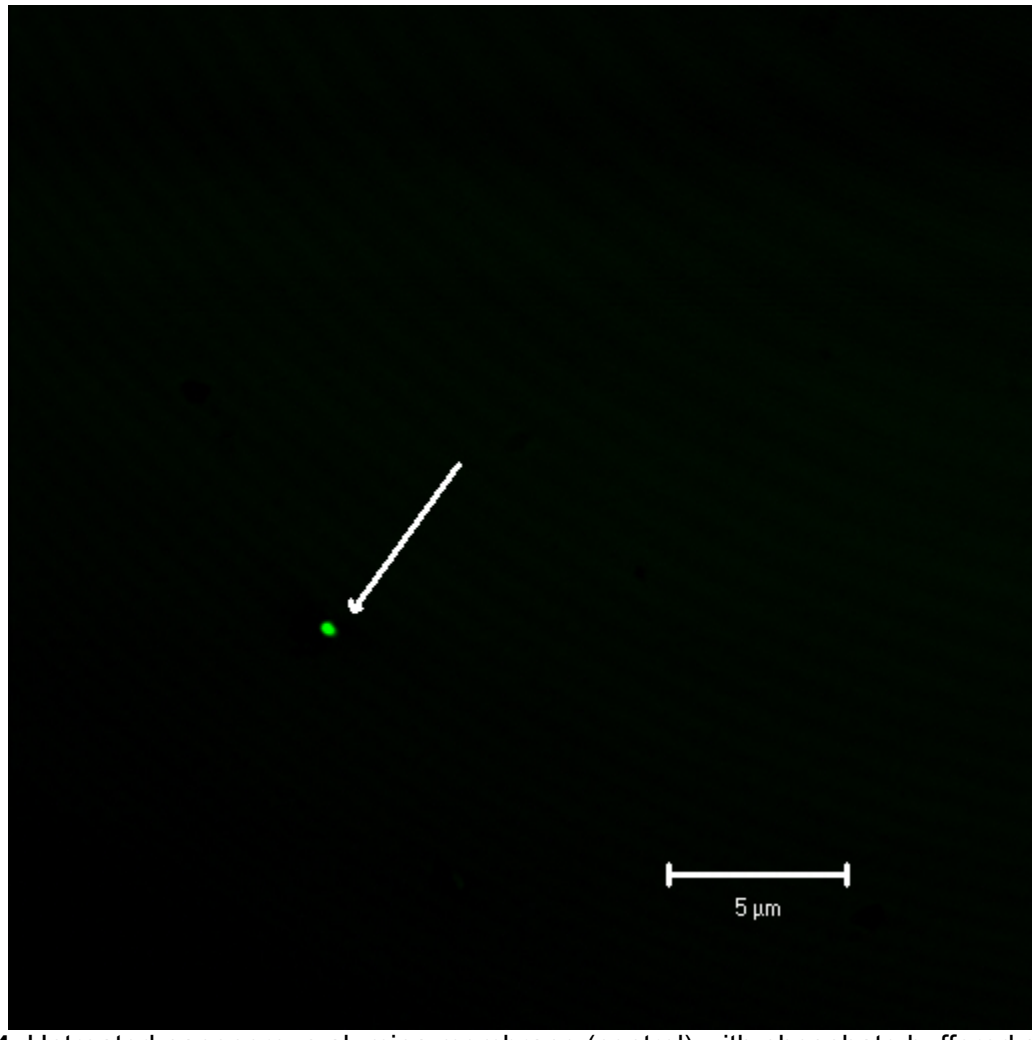

Figure 4. Untreated nanoporous alumina membrane (control) with phosphate buffered saline (PBS) solution, demonstrating as few as one cell/field.

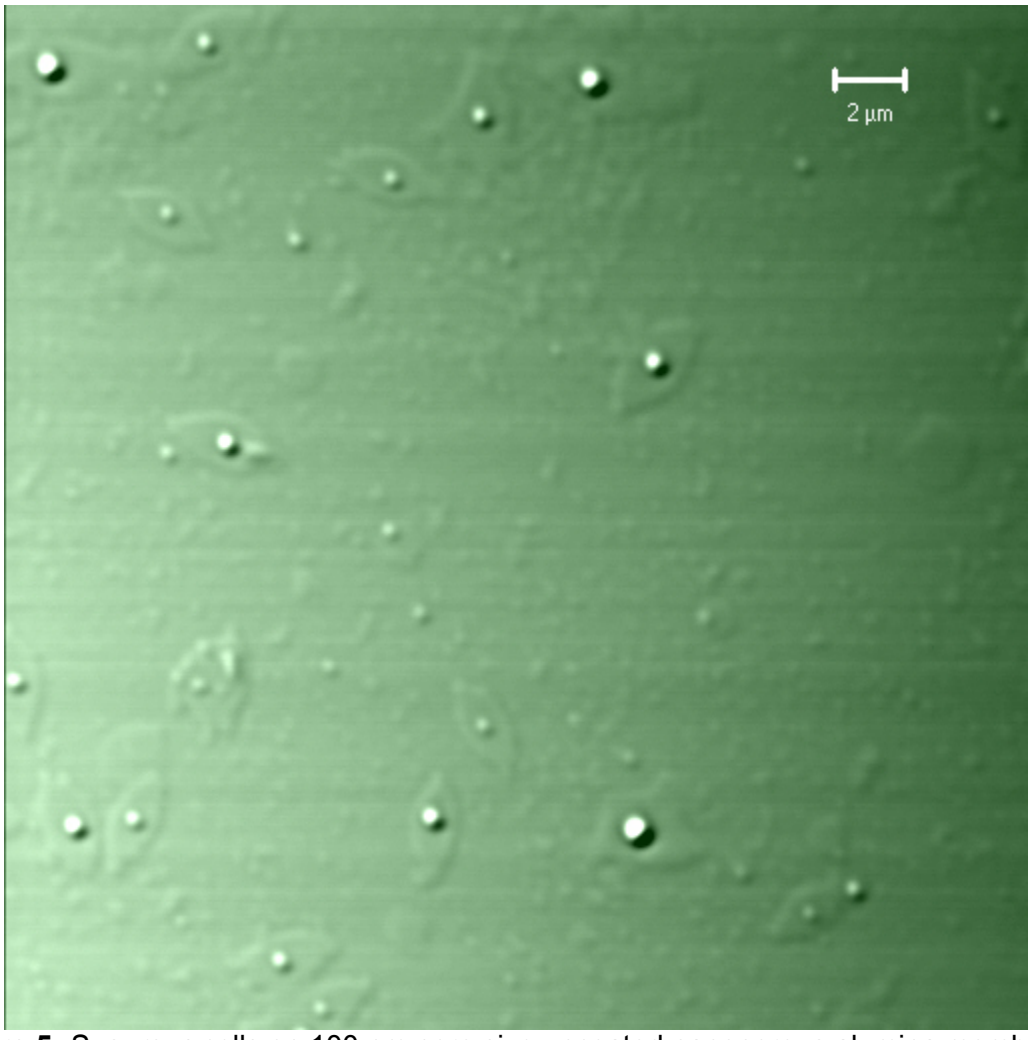

Figure 5. *S. aureus* cells on 100 nm pore size uncoated nanoporous alumina membrane.

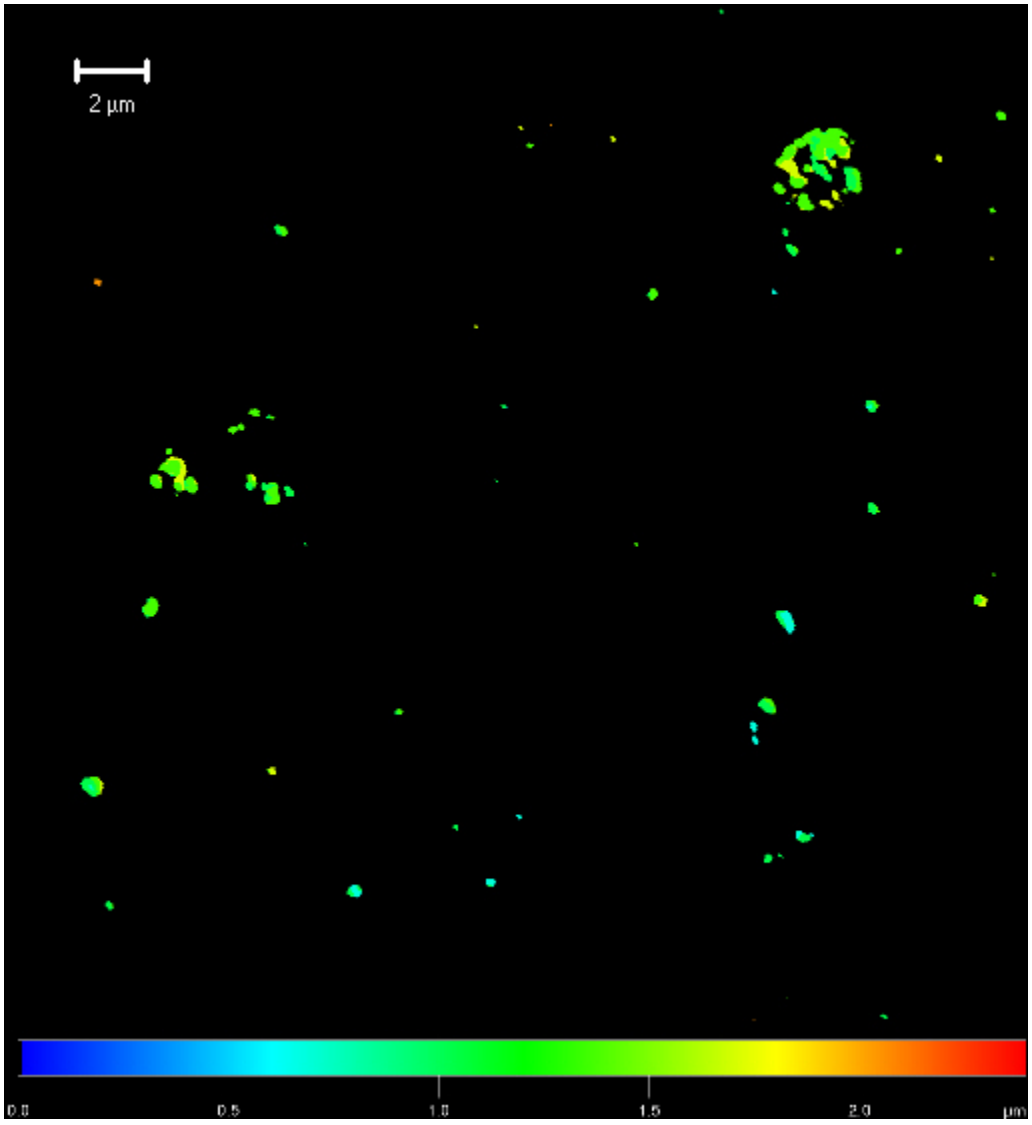

Figure 6. Three dimensional view showing some *E. coli* cell aggregates on a 20 nm pore size uncoated nanoporous alumina membrane.

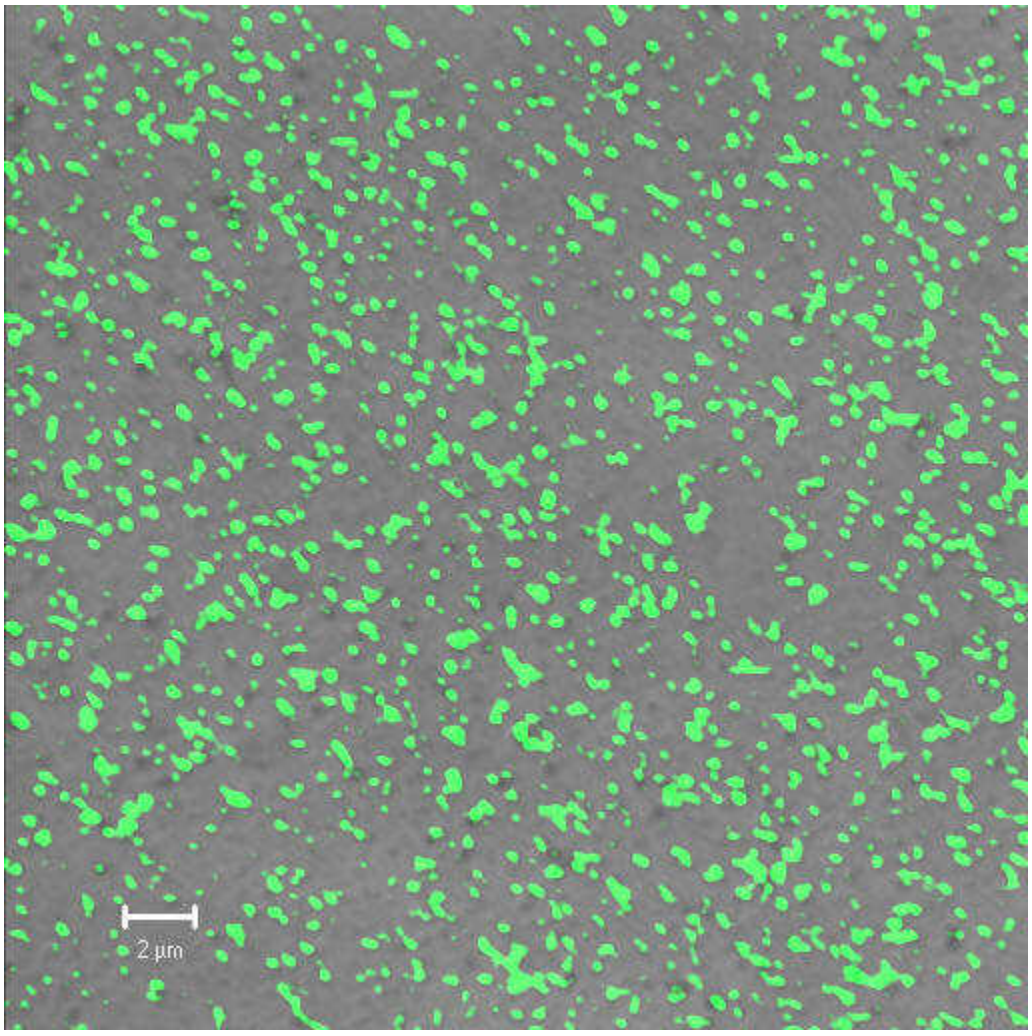

Figure 7. High densities of *S. aureus* observed on 100 nm pore size titanium oxide-coated nanoporous alumina membrane.

Table 1. Microbial densities on slides after treatment

Treatment	Membrane	Membrane type	Bacteria Density*
No bacteria (PBS)	uncoated	20 nm	1 cells/field
No Bacteria (PBS)	uncoated	100 nm	3 cells/field
S. aureus	uncoated	20 nm	340 cells/field
E. coli	uncoated	20 nm	67 cells/field
S. aureus	uncoated	100 nm	322 cells/field
E. coli	uncoated	100 nm	71 cells/field
S. aureus	4.3 nm titanium oxide	20 nm	8 cells/field
E. coli	4.3 nm titanium oxide	20 nm	12 cells/field
S. aureus	8.6 nm titanium oxide	100 nm	27 cells/field
E. coli	8.6 nm titanium oxide	100 nm	156 cells/field

*Densities reported are the average of 10 fields counted on each membrane