More Efficient Capture of Bacteria on Nanophase Materials

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Introduction

Nanobiotechnology is a growing area of research [1], primarily due to the potentially numerous applications of new synthetic nanomaterials in engineering/science. Although various definitions have been given to the word "nanomaterials" by many different experts, the commonly accepted one refers nanomaterials as those materials which possess grains, particles, fibers, or other constituent components that have one dimension specifically less than 100 nm [2]. Such novel surface properties contribute to the noted increased wettability of nanophase compared to conventional ceramics and, consequently, have lead to the investigation of nanophase materials in biological (or aqueous) environments. In biological applications, most of the research to date has focused on the interactions between mammalian cells and synthetic nanophase surfaces for the creation of better tissue engineering materials [3-8]. Although mammalian cells have shown a definite positive response to nanophase materials [3-8], the evidence for bacteria interactions with nanophase materials remains at large a mystery. For these reasons, the objective of the present study was to determine the interactions of one model bacteria (*Pseudomonas fluorescens*) on nanophase compared to conventional alumina substrates.

Experimental

Materials

As a model nanophase material, alumina was used in the present study. Nanophase alumina (23 nm size) particles were obtained from Nanophase Technologies, Corp. (Romeoville, IL). Nanophase ceramic particles were used as supplied by the manufacturer. Nanophase alumina particles were first loaded into a steel-tool die to obtain compacts for use in bacteria experiments. A serial pressure cycle (from 0 - 10 GPa over 11 minutes) using a simple uniaxial, single-ended hydraulic press (Carver, Inc.) was utilized to press all alumina particles into compacts. Particles were pressed in air at room temperature. After pressing, nanophase alumina was heated (in air at 10°C/min.) to 800°C and sintered at this temperature for 2 hours. Sintering at this temperature created crystalline alumina with nanometer grain sizes. To obtain larger conventional grain size (i.e., control) compacts, alumina nanophase compacts were heated (in air at 10°C/min.) to 1200°C and sintered at this temperature for 2 hours. Compacts were prepared with an 11 mm diameter, and were fixed to the center of a glass slide (Fisher Scientific) with silicon-based sealant. Compacts were rinsed with sterile deionized, distilled water and were then autoclaved before use in cell culture experiments.

Material Characterization

The purpose of the following material characterization techniques was to create and test nanophase and conventional alumina compacts that altered only in grain size but were of the same bulk crystallinity, chemistry, crystal phase, and porosity. That is, a focused study was

designed to largely examine bacteria response to changes in nanometer compared to conventional topographies; such studies are often lacking in the examination of material interactions with living organisms.

Ceramic compact crystallinity was examined using X-ray diffraction (Phillips type PW2273/20). Nickel filtered copper Kα radiation (λ = 1.5406 nm) produced at 40 kV and 35 mA was used to scan the diffraction angles (2θ) between 30° and 35° at every 0.02° for 20 seconds/angle. Diffraction signal intensity throughout the scan was monitored and processed using Scintag (Sunnyvale, CA) DMS software. Ceramic particle size was determined through multiple point BET measurements using an SA 3100 gas adsorption analyzer (Beckman Coulter, Inc.) according to manufacturer's instructions.

Bacteria Interactions

To determine bacteria response to nanophase compared to conventional alumina, a pure culture of *Pseudomonas fluorescens* MF0 was donated by Professor Sylvie Chevalier (Université de Rouen, Evreux, France) and was used as a model bacteria cell line in these experiments. *P. fluorescens* is ubiquitously distributed in water and soil, and is frequently isolated from environmental and food specimens. Pure cultures of *P. fluorescens* MF0 were grown at room temperature (22 ± 0.5 ºC) with continuous shaking at 150 rpm in trypicase soy broth (Difco). Cultures were harvested when the optical density at 600 nm was approximately 1.00, which was determined to be the mid-log phase for this strain. The optical density was determined using a Perkin Elmer Lamda 20 Spectrometer (Perkin Elmer Inc., USA). The cultures were harvested by four consecutive stages of centrifugation (6000g for 10 min and at 4 °C) and re-suspended in phosphate buffer saline (PBS) (pH 7.4, 137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, and 2 mM KH_2PO_4). The final suspensions from centrifugation vials were combined to a volume of 300 ml with cell concentrations equal to approximately 5×10^8 cells/mL.

For each bacteria capture batch assay, alumina compacts of the two topographies (either nanophase or conventional) were tested in parallel. Four treatments were assessed (nanospherical alumina without fibronectin, nanospherical alumina with fibronectin, conventional alumina without fibronectin, and conventional alumina with fibronectin), along with appropriate controls. Fibronectin is an adhesive protein that mediates *P. fluorescens* capture. Alumina compacts were coated with 5 μg/ml of fibronectin (Sigma) in PBS for 8 hours overnight at room temperature. The capture experiments were carried out in triplicate in 50 mL centrifuge vials. First, 50 mL of the centrifuged bacterial suspension in PBS (or filtered, sterile water for the control) was placed into each vial. Then, a glass slide containing either nanophase or conventional alumina was gently inserted into the vial. Caps were secured tightly on the vials to prevent leakage. The vials were immediately placed on a slantwise rotator and slowly rotated at 12 rpm. After 1 hour, the vials were opened and the adhesion of bacteria onto the surface was assessed by epi-fluorescence microscopy. Bacterial cell counts were conducted with a Nikon E 800 Bio-Research Microscope (Nikon Instrument, Inc. USA) at 1000x magnification. Approximately 15 different fields of 0.01 mm^2 for each sample were randomly selected to allow for statistical significance. Bacteria capture was normalized to alumina compact surface area. Experiments were run in triplicate at repeated at least three separate times. All data was analyzed by standard t-tests with statistically differences between means determined at $p < 0.05$.

Results and Discussion

Material Characterization

Although not measured at the atomic level, results of this study provided evidence that the nanophase and conventional (that is, particle sizes larger than 100 nm) alumina compacts possessed similar chemistry, crystalline phase, no-porosity, and altered primarily in surface feature size or degree of nanometer surface roughness. Specifically, mean particle diameters as quoted by the supplier (Nanophase Technologies, Corp.) were confirmed at 23 nm for the alumina nanophase particles. In contrast, conventional alumina particle diameters were 179 nm. Results also provided evidence of similar crystalline phases between nanophase and conventional alumina (γ phase).

Increased Bacteria Cell Capture on Nanophase Alumina

Most importantly, the results of this study provided the first evidence of increased bacteria cell capture on alumina that alters only in degree of nanometer surface roughness. Specifically, the nanophase alumina surface captured significantly ($p < 0.01$) higher *P. fluorescens* than the conventional surface either in the presence or absence of adsorbed fibronectin. In fact, 2 and 1.4 times the amount of *P. fluorescens* were captured on nanophase compared to conventional alumina with and without pre-adsorbed fibronectin, respectively. The surface with the highest level of adhesion was the nanophase alumina with pre-adsorbed fibronectin. There was no significant difference between the conventional surface with pre-adsorbed fibronectin and the nanophase surface without pre-adsorbed fibronectin. The conventional surface without fibronectin had the lowest level of bacterial adhesion.

Conclusions

In summary, although there has been speculation about bacterial interactions with nanophase materials, very little research has been reported in this area. In this manner, this study represents one of the first (if not the first) to demonstrate the benefits of using nanophase materials in environmental applications for the efficient capture of bacteria from waste water, soil, etc.

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