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Responses of *Staphylococcus aureus* bacterial cells to nanocrystalline nickel nanostructures

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ABSTRACT

A broad range of human diseases are associated with bacterial infections, often initiated by specific adhesion of a bacterium to the target environment. Despite the significant role of bacterial adhesion in human infectious diseases, details and mechanisms of bacterial adhesion have remained elusive. Herein, we study the physical interactions between *Staphylococcus aureus*, a type of micro-organism relevant to infections associated with medical implants, and nanocrystalline (nc) nickel nanostructures with various columnar features, including solid core, hollow, x-shaped and c-shaped pillars. Scanning electron microscopy results show the tendency of these bacterial cells to attach to the nickel nanostructures. Moreover, unique single bacterium attachment characteristics were observed on nickel nanostructures with dimensions comparable to the size of a single bacterium.

1. Introduction

Staphylococcus aureus (*S. aureus*) is a grape-like shaped bacterium that can adhere to organic [1–3] and metal [1,2,4–10] surfaces. The overall geometry of *S. aureus* is round ("coccus") with diameters approximately ~0.5 μm. This bacterium is a common source of nosocomial infections especially after implant associated surgeries [6,11], such as prosthetic joint implants [12], and heart valves [13]. In addition, they are a common cause of food borne illnesses by adhering to food service surfaces and contaminating food supplies [14].

Moreover, the recent discovery of drug resistance strains of *S. aureus* [15–17], such as methicillin-resistant and oxacillin-resistance *S. aureus*, has led to an emergence of research on bacterial adhesion and survival mechanisms on various surfaces. Several experimental surface coatings and treatment techniques on implant surfaces have been developed [7,9,18–22] with the goal of enhancing osseointegration and reducing bacterial cell adhesion capabilities. Recent studies have suggested a sensitivity of bacteria to nanoscale topographical properties of implant substrates. Wu et al. [23] performed an *in vitro* study on the effects of titanium surface roughness on *Staphylococcus epidermidis* and human osteoblast behavior. These surfaces were prepared with polished, satin, grit-blasted and plasma-sprayed surface finishes. Their results indicated that not only the vertical roughness is important but also the lateral roughness parameters of these small surface features play a role in bacteria attachment. Furthermore, their results showed a preferential colonization of bacteria on micro-rough surfaces, whereas the osteoblasts favored interaction with smooth plasma-sprayed surfaces than with rough satin treated titanium substrates. In a different study, Truong et al. [24] highlighted the effects of nanoscale surface roughness on the adhesion of *S. aureus* and *Pseudomonas aeruginosa* bacteria. Bacteria attachment densities were compared on substrates with different topographical features, but with identical surface chemistry and wettability. Their experiments demonstrated altered bacteria adhesion merely due to surface nano-topography. Specifically, they showed that *S. aureus* tend to attach at significantly higher densities to treated ultrafine-

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grained titanium substrates as compared to untreated bulk substrates. Truong et al. [24] hypothesized that surface nanotopography is a leading factor in controlling bacterial attachment. The effects of nanorough, nanotubular, and nanotextured titanium surfaces on *S. aureus* adhesion were also investigated by Puckett et al. [25]. They demonstrated that bacteria are less likely to adhere to the nanorough Ti surfaces prepared with electron beam evaporation but prefer to attach to nanotubular surfaces.

Despite the industrial and clinical importance of the bacterial surface adhesion, there is no in-depth study on single *S. aureus* cell interactions with well-defined nanometer scale three-dimensional structures. Herein, we present a detailed investigation aimed at understanding how *S. aureus* cells attach to nanocrystalline (nc) nickel columnar nanostructures with various 3D nano-topographical features. These nanostructures include pillars of various cross-sectional geometries, namely solid core, hollow, c-shaped, and x-shaped pillars. These features have outer diameters as small as 220 nm. Three-dimensional mushroom shaped nanostructures were also prepared to understand how these cells interact with overhang topographies. These complex nanostructures were fabricated with electronic beam lithographic techniques and electroplating methods (see Experimental methods). Finally, high-resolution scanning electron microscopy was used to explore the behaviors of individual *S. aureus* cells on these nanometer scale metallic nanostructures of various 3D topographic features comparable with the size of a bacterium.

2. Experimental methods

Nanometer scale nanocrystalline nickel pillars were prepared by using state-of-the-art electronic beam lithographic (EBL) methods and electroplating techniques [26–30]. Fig. 1 illustrates the fabrication steps for these nanostructures. Thin titanium (~20 nm) and gold (~100 nm) films were first deposited on bare silicon substrates using

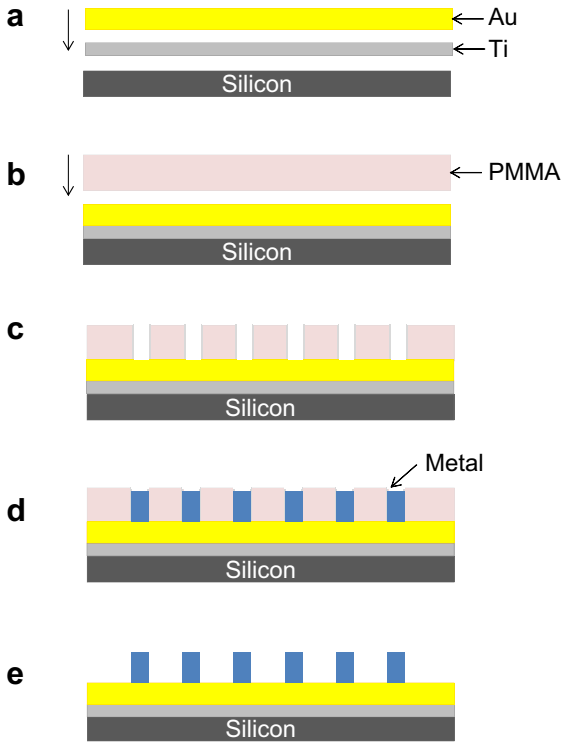

Fig. 1. Nanostructure fabrication process a) electron beam evaporation of 20 nm titanium and 30 nm gold on silicon substrate, b) spin coating of PMMA resist, c) patterning of PMMA resist with ebeam lithography, d) electroplating of desired metal into patterned holes, e) PMMA resist removal via acetone.

electron beam deposition methods. They are then spin coated with Poly (methyl methacrylate) (PMMA) EBL resists. Complex geometric via-hole patterns were produced on these silicon wafers by exposing these thermal plastic films to an electron beam. Nanocrystalline nickel was then deposited in these hole patterns with an electrolyte that contains nickel (II) sulfate hexahydrate (99%, Sigma Aldrich), nickel (II) chloride (98%, Sigma Aldrich), and boric acid (BX0865, EMD Millipore) with concentrations of 300 g/L, 30 g/L, and 30 g/L, respectively. A small amount of saccharine (1.9 g/L) was also added in the plating solution in order to reduce the nickel crystalline size while the current density was maintained at 11.5 ± 2 mA/cm^2 during the plating process. The excess PMMA film was dissolved in acetone after the nickel deposition processes. Detailed transmission electron microscopy analyses revealed a grain size in the range of 9.4 and 13.2 nm for these nickel nanostructures [30].

S. aureus (ATCC 6538) bacteria were generously provided by Dr. Lyndon Jones' laboratory at the University of Waterloo. *S. aureus* bacteria were cultured on trypticase soy agar (TSA) plates by using alginate swabs and incubating the plates at 37 °C overnight. A 2.55% saline solution was prepared and sterilized by using Nalgene filters and ~0.006% of nutrient broth was added to the saline to preserve *S. aureus* during tests. *S. aureus* cells were transferred to saline solution by adding 5 mL of saline to the TSA plate and using alginate swabs to dislodge the bacteria from the plates. *S. aureus* cells were washed with saline solution seven times by centrifugation at 4000 rpm for 10 min. The stock solution of *S. aureus* cells was diluted 10-fold in saline before testing. During a typical test, a drop of diluted *S. aureus* solution was placed on the silicon substrate containing nc-nickel shaped pillars. The specimens were left in the incubator with constant temperatures of ~37 °C. After 6 h, the samples were rinsed with deionized (DI) water to remove cells that are not well adhered to the surfaces and air dried in fume hood for 12 h. Field emission scanning electron beam microscope (Zeiss LEO 1550) was used to inspect how these *S. aureus* cells interact with nickel nanostructures.

3. Results and discussions

Representative 70° tilted scanning electron microscopy (SEM) images of as-fabricated nc-nickel nanostructures with solid core, hollow, c-shaped, and x-shaped pillars are shown in Fig. 2(a–d), respectively. The outer diameter of these vertical pillars is ~1000 nm with a height to diameter aspect ratio of approximately 1.5. Fig. 2(b) shows a representative image of the hollow pillars with average inner diameters of ~840 nm. The c-shaped pillars have an inner diameter of ~760 nm (see Fig. 2(c)). The small openings along the edges of c-shaped pillars allow inspections of the interiors of these nanostructures. Careful SEM inspections of the fabricated pillars with different shapes reveal the pillar exterior sidewalls are extremely smooth and aligned nearly perfectly vertical from the substrate surface. Furthermore, the top surfaces of these nanostructures are flat and slightly rougher than the sidewalls but the roughness still remains in the nanometer scale.

All the nanostructures shown in Fig. 2 were fabricated simultaneously on a single silicon substrate, and are thereby expected to have similar surface chemical compositions and wettability, and differ only in nanometer scale morphology. Furthermore, cell plating was carried out on a single substrate containing all pillar shapes under identical environmental treatments. These nanocrystalline nickel pillars are regularly spaced at a 10 μm center to center distance as shown in Fig. 2(f). In order to better examine how an individual *S. aureus* cell interacts with overhang nano topography, mushroom shaped nc-nickel nanostructures with stem diameters of 220 nm were fabricated as displayed in Fig. 2(e). The micrographs clearly reveal the smooth nickel pillar sidewalls.