Preparation of a Nano-patterned Polymer Replica for Reducing Catheter-associated Infections

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Statement of Purpose: Nowadays, catheter-associated infections are the most serious and costly of all health-associated infections. [1] Recent studies have suggested a sensitivity of cell and bacteria to nano-scale topographical properties as a potential tool for selectively increasing desirable cell functions while simultaneously decreasing competitive cell functions. [2] Here, we presented a simple and cheap method to prepare a nano-patterned polydimethylsiloxane (PDMS, a commonly used catheter material) replica by using highly ordered nanotubular anodized titanium (ATi) as the template. In vitro bacterial studies using *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* were conducted to assess the effectiveness of the nano-PDMS at inhibiting bacterial growth. In addition, human fibroblast and endothelial cell assays were conducted to determine the influence of the nano-patterned structure on mammalian cell behavior as a measurement of toxicity. In this study, to elucidate the mechanisms of how surface topographies affect cell/ bacteria adhesion, the protein interactions with different surfaces were also investigated by using the bicinchoninic acid (BCA) protein assay.

Methods: For anodization, an etched Ti sample was used as an anode, while a platinum (Pt) mesh served as a cathode. Both were immersed in an electrolyte solution consisting of 1.5% hydrofluoric acid and were connected to a DC power supply. Next, the PDMS slurry was cast onto the ATi template and then was placed into a vacuum chamber for 1h. The PDMS sample was cured at 60 °C for 2h followed by cooling and was gently peeled away from the Ti template.

S. aureus, *E. coli*, *P. aeruginosa* and *S.epidermidis* (ATCC 25923, 25922, 31479 and 35983, respectively) were used in this study. Sterile samples were treated with the prepared bacterial solution ($\times 10^7$) and cultured for either 24h or 48h in an incubator (37°C, 5% CO_2, humidified). Afterwards, the samples were rinsed with a 10mg/mL PBS solution and placed into 15 mL tubes with 4 mL PBS. These tubes were shaken at 3000 rpm for 15 minutes on a vortex mixer to release the bacteria attached on the surface into the solution. Solutions with bacteria were spread onto agar plates and bacteria colonies were counted after 18h of incubation. Human fibroblasts (ATCC CCL-110) and endothelial cells (Life Tech) at population numbers less than seven were used for all cell experiments. Cells were seeded onto the substrates at a density of 5000 cells/cm^2 and were allowed to adhere for 4 h in a 37°C, humidified 5% CO_2 atmosphere. MTT assays were used in this study. All the tests were performed in triplicate and repeated three times.

BCA Protein assay reagents (Thermo Scientific) were used to measure the total protein concentration on different samples according to the standard instructions.

Results: As expected, the nano-sized tubes were distributed uniformly on the Ti surface after anodization and nano-patterned structures were fabricated successfully on the surface of PDMS by using ATi as the template. The contact angles results highlighted that upon nanostructuring the PDMS surface, it became slightly more hydrophilic. It was found that nano-patterned PDMS templates increased both fibroblast and endothelial cell adhesion and, thus, were non-toxic to mammalian cells. Results also showed that nano-patterned PDMS inhibited the growth of all the four types of bacteria after 24h and 48h, respectively (Fig. 1). Moreover, data suggested the effectiveness of bacteria inhibition reached above 50%, all without employing an antibiotic. Therefore, the presently fabricated nano-patterned PDMS surfaces could be a promising way to fight catheter-associated infections

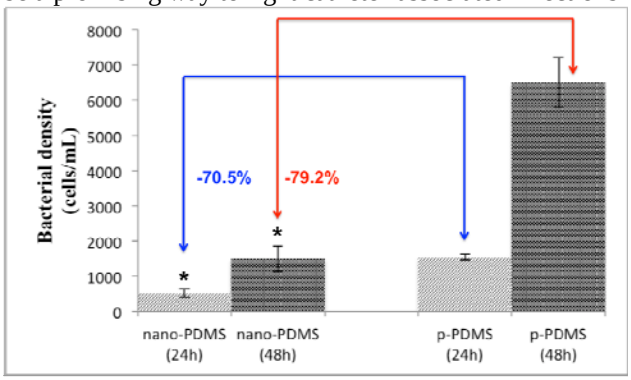

Figure 1. *S. aureus* growth on the surface of nano-patterned PDMS (nano-PDMS) and plain PDMS (p-PDMS). Data represents mean ± SD, n=3. *p < 0.05 compared with p-PDMS at the same time period.

Conclusions: Nano-patterned structures were successfully fabricated on the surface of PDMS through an ATi template method. Furthermore, the nano topography on PDMS could inhibit bacterial growth significantly while remaining non-toxic to mammalian cells, and thus should strongly be considered for use in catheters.

Acknowledgements: The author would like to thank the Webster Nanomedicine Lab and the Department of Chemical Engineering, Northeastern University for funding.

References:

[1] Noimark S, Dunnil CW, Wilson M, Parkin IP. The Role of Surface in Catheter-associated Infections. Chemical Society Reviews 2009; 38:3435-3448.

[2] Ercan B, Taylor E, Alpaslan E, Webster TJ. Diameter of Titanium Nanotubes Influences Anti-bacterial Efficacy. Nanotechnology 2011; 22:295102.