Preparation of a Nanopatterned Polymer Replica for Reduced Catheter Inflammation and Infection

Luting Liu, Batur Ercan, Siyu Ni, Linlin Sun and Thomas J. Webster
Department of Chemical Engineering, Northeastern University, Boston, MA

Statement of Purpose:

Inflammation and infection of catheters (and polymeric medical devices in general) are significant problems. It is hypothesized that nanostructured surfaces can be carefully manipulated to inhibit immune cell (e.g., macrophages) and bacteria responses due to their unique surface energy properties which have the ability to control initial protein absorption and subsequent cell behavior. [1] The objective of this in vitro study was to create nanopatterned polydimethylsiloxane (PDMS) molds based on anodized titanium (Ti) and anodized stainless steel and then to test inflammatory cell and fibroblast responses on such substrates.

Methods:

To prepare the nanopatterned master, 2.5 cm^2 titanium foils (Alfa Aesar, Ward Hills, MA) were cleaned with acetone, 70% ethanol, deionized water and were then etched for 1min with a solution of 1.5% nitric acid and 1.5% hydrofluoric acid (HF) to remove the thin oxidized layer. The cleaned 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% HF and were connected to a DC power supply.[2] The similar method is applied on 316 stainless steel in ethylene glycol containing 10% perchloric acid.

Next, the PDMS monomer and cross-linking agents (Sylgard 184, Dow Chemical Co.) were mixed at 10:1 for 15min and were then placed in a vacuum chamber for 30min to remove air bubbles. The mixture was cast onto the nanopatterned master mold and then was placed into a vacuum chamber for another 1h. The desired PDMS replica was cured at 60 °C for 2h followed by cooling and was gently peeled away from the master. [3]

To determine the adhesion and proliferation of skin fibroblasts (ATCC, CCL-110), a cell proliferation assay (CellTiter 96, Promega) was used. Briefly, for cell adhesion, cells were seeded at 3500 cells/cm^2 in standard cell culture media and were incubated for 4 hours. For proliferation studies, cells were seeded at 3500 cells/cm^2 for 1, 3 and 5 days. The dye solution were added to the cells after the end of the prescribed period for 4h, then the stop solution was added and incubated overnight. A plate reader was used to test cell density.

Also, the IC-21 macrophage cell line was used (TIB-186; ATCC, Manassas, VA) for determining an immune response. Macrophages were cultured according to ATCC instructions in RPMI-1640 medium (Invitrogen, Carlsbad, CA) with 10% fetal bovine serum and 1% penicillin/streptomycin (HyClone Laboratories Inc, Logan, UT) under standard incubator conditions (37°C, humidified, 5% CO_2/95% air environment). Macrophages were seeded at 3500 cells/cm^2 per substrate and were cultured in a humidified 5% CO_2/95% air environment at 37°C for 24 hours. After 24 hours, nonadherent macrophages were removed by rinsing in phosphate-buffered saline (PBS). Macrophages adherent on the substrates were fixed with 4% formalin in PBS and stained with both rhodamine phalloidin (R415) (Molecular Probes, Eugene, OR) and DAPI (4', 6-diamidino-2-phenylindole) (33258) (Sigma-Aldrich). F-actin filaments and cell nuclei were visualized using tetramethyl rhodamine iso-thiocyanate and DAPI, respectively.

Results:

As expected, nano-sized tubes were distributed uniformly on the titanium surface after anodization. When anodization was carried out for 10min at 5V, 10V, 15V, 20V resulted in nanotubes with 20nm, 40nm, 60nm, 80nm diameters, respectively. And a nano-porous structure was also seen from anodized Stainless steel at 40V with the pore size of 50nm. After pouring the PDMS slurry onto the surface of masters, a nanopatterned structure was observed. Most importantly, the PDMS replica showed similar nanostructures as anodized Ti and Stainless Steel (Figure 1).

The results also demonstrated decreased numbers of macrophages adhered to nanotubular Ti and PDMS replica surfaces. Most importantly, the increased fibroblast adhesion, proliferation, and long-term functions on nanotubular substrates indicated the nanoscale topographies of polymers should be strongly considered for catheter applications.

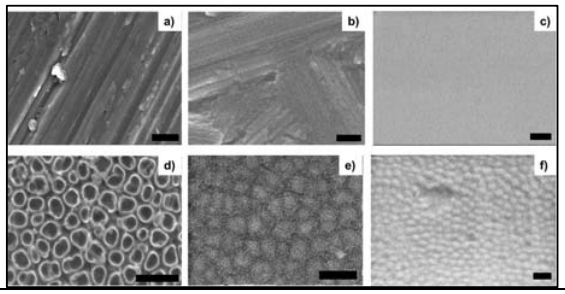

Figure 1. SEM micrographs of: (a) unanodized Ti; (b) PDMS on unanodized Ti; (c) Pure PDMS; (d) Anodized Ti; (e) PDMS on anodized Ti; (f) PDMS on anodized Stainless steel. Scale bars are 200nm.

Conclusions:

A nano patterned structure was successfully fabricated on the surface of PDMS through anodized masters and demonstrated a promising ability to control macrophage and fibroblast functions.

Acknowledgements:

The authors would like to thank all Webster nanomedicine lab members for help and Northeastern University for funding.

References:

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[3] H. Hassanin, et al. Lab chip. 2012; 12(20): 4160-4167.