Nanopost Fence: A Novel Strategy of Preventing Smooth Muscle Cells Topographic Migration

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Statement of Purpose:
Smooth muscle cell migration is a vital mechanistic process involved in atherosclerosis, restenosis, and general vascular response to injury[1]. In the native artery, the internal elastic lamina is the natural defense against cell migration[2]. Here, we attempt to emulate the topographic defense of the internal elastic lamina by virtue of a nanostructure strategy by using electron beam lithography. We created a polyethylene glycol (PEG) nanopost fence at varying depth and spatial configurations to define a proper density such that linear smooth muscle cell (SMC) migration across a given zone can be prevented. We hypothesize that at a defined density will provide a physical sets-like-barrier through which SMC cannot physically migrate and transgress.

Methods:
Polished stainless steel was laser etched into 1x1 cm chips. The chip surface was cleaned with acetone, followed by isopropyl alcohol in clean room. The chips were then plasma treated for 10 minutes at a pressure of 1 Torr. Following plasma treatment, the chips were spun coat with a layer of 3.2% (w/v) PEG in methanol at 3000 rpm in a light sensitive environment. The chips were then patterned using electron beam lithography with a beam current of 30 keV and a spot size of 3.5. The patterns were created using DesignCad Express v.16 and were relayed to the electron beam using NPGS. Once the patterns were completed, the unexposed PEG was rinsed away with water, leaving only the crosslinked nanopost. The height and base profile of the posts were obtained using atomic force microscopy (AFM) in tapping mode. The chips were then subjected to cell media at 5, 12, and 20 dyne/cm^2 for upto 72 hours to determine the degradability of PEG nanopost by using the the parallel flow plate chamber and AFM. Chips were also cultured with cells for 72 hours statically and subsequently fixed with glutaraldehyde, dried with series of EtOH (25%:50%;75:100%) and finally subjected to critical point drying and scanning electron microscope (SEM).

Results:
It is shown through our fabrication method that the PEG nanopost fence can be arranged in specific sizes, patterns, and densities (Figure 1A,C). Although some degradation of the posts occurred, it was shown that they retained the core of their structural profile when placed under flow-induced shear stresses. These findings were consistent across different array patterns and chips over the course of three days (Figure 1B). Our preliminary results suggest that we can barricade cells from physical migration and transgression toward a given zone (Figure 1D).

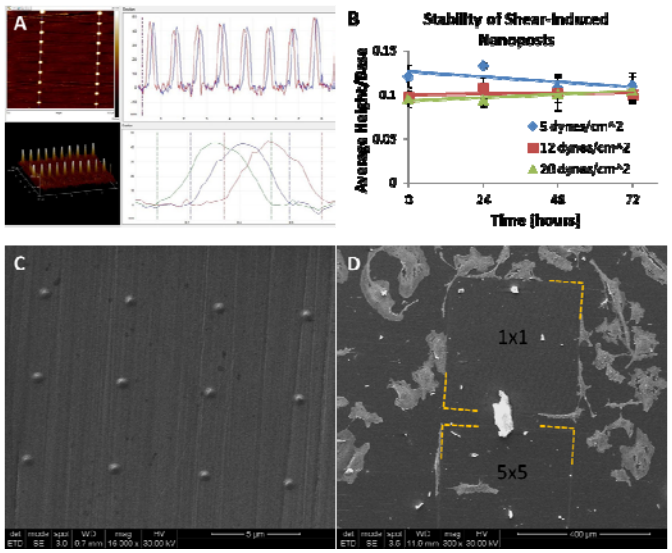

Figure 1. Characterization of nanopost and barrier zone. A) AFM images of nanopost showing sectional height and base dimension. B) Stability of shear-induced nanopost after 24, 48, and 72 hours of cell culture media exposure. C) SEM of nanopost at 5x5 µm. D) Barrier zone preventing cells from physical migration and transgression.

Conclusions:
We found that PEG nanopost crosslinked by EBL can withstand degradation under static and shear-induced environments for up to 72 hours. The nanopost density at 1x1 µm so far seems to be the best physical barrier. Using a nanopost fence as a physical barrier in combination with antiproliferative drugs and cyclic ligands would add another modality to therapeutically prevent neointimal thickening.

References:
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2. Ajello VD, Gutierrez PS, Chaves MJ, Lopes AA, Higuchi ML, Ramires JA. Mod Pathol. 2003, 16(5):411-416.