**Photo-crosslinked Polymer Micro-pillar Arrays to Control Smooth Muscle Cells**

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**Statement of Purpose:** Vascular smooth muscle cells (SMCs) are sensitive to the topographical features of the extracellular matrix (ECM) through the sensing molecules in cell membrane. The desirable landscape of ECM at the micron, submicron, and even nanometer scales attracts vascular cell adhesion to the surface, promotes cell proliferation and differentiation, and supports formation of functional blood vessels. Here we fabricated arrays of cylindrical pillars with three heights of 3.4, 7.4, and 15.1 μm by photo-crosslinking poly(ε-caprolactone) triacrylate (PCLTA) in silicon molds with pre-designed micropatterns. Then we studied SMC adhesion, spreading, elongation, proliferation, and differentiation on these substrates with the micro-pillar arrays.

**Methods:** Ring-opening polymerization of ε-caprolactone into PCL triol was initiated by 1,1,1-tris(hydroxymethyl) propane in the presence of Sn(Oct)2 as the catalyst. Crosslinkable PCLTAs were further synthesized through acrylation of PCL triol in the presence of potassium carbonate as the proton scavenger [1-3]. Phenyl bis(2,4,6-trimethyl benzoyl) phosphine oxide (BAPO, IRGACURE819, Ciba Specialty Chemicals, NY) was used as the photo-initiator in crosslinking PCLTAs. The solution of 1.5 g PCLTA in 500 μL CH2Cl2 was mixed with 75 μL of BAPO/CH2Cl2 (300 mg/1.5 mL) solution as the resin for photo-crosslinking. The silicon molds for producing micro-pillar heights of 3.4, 7.4 and 15.1 μm were prepared through standard microfabrication procedures. Then the PCLTA/BAPO/CH2Cl2 mixture were poured onto the silicon molds and photo-crosslinked for 30 min under a UV lamp (SB-100P, Spectroline, wavelength = 365 nm, intensity = 4800 μW/cm²) (Fig. 1a) [1-3]. In cell studies, primary SMCs isolated from rat aorta were cultured in DMEM with 10% fetal bovine serum on photo-crosslinked PCLTA substrates at a density of ~15,000 cells/cm².

**Results:** The surface patterns of the substrates with micro-pillar arrays were characterized using Scanning Electron Microscopy (SEM, Fig. 1b). For all the three heights, the micro-pillar diameters and the inter-pillar distance were determined to be 3.7-3.8 and 2.0 μm, respectively. Substrate hydrophilicity was characterized using the water contact angles shown in Fig. 1c. The water contact angles were higher when there were micro-pillar arrays on the substrates for both crosslinked PCLTA7k and PCLTA10k, especially when the micro-pillars were longer. SMCs on flat crosslinked PCLTA7k were better spread than those on the micro-pillar arrays, especially when the micro-pillars were longer. As shown in Fig. 1d, SMCs on the flat crosslinked PCLTA7k substrate had regular distribution of filaments, whereas the cells on the micro-pillar arrays showed bright dots around micro-pillars and dark dots on the micro-pillar points. This result indicated that cytoplasm was largely developed in the inter-pillar spaces with a small portion on the pillar top. To better illustrate the scenarios, schemes are shown in Fig. 1d. SMCs cultured on the flat substrate developed large, well-elongated focal adhesions. In contrast, SMCs cultured on the micro-pillar substrates were only able to develop weaker, irregular focal adhesions. SMC nuclei at day 1 on the flat substrates were round with a high fluorescence intensity while those on the micro-pillar substrates were smaller with weaker fluorescence and the nuclear size decreased with increasing the pillar height.

**Conclusions:** Photo-crosslinked PCLTA substrates with micro-pillar arrays facilitate SMC attachment and elongation but they inhibit SMC spreading and proliferation. SMC nuclei were smaller on the micro-pillar substrates than on the flat ones. SMC filaments and focal adhesions had intensive distribution around the micro-pillars. The gene expression levels of four contractile markers in SMCs cultured on the micro-pillar substrates were all higher than those on the flat ones, suggesting that micro-pillars could facilitate the conversion from the proliferating synthetic phenotype to the functional contractile phenotype.

**Acknowledgements:** University of Tennessee and NSF (DMR-11-06142).

**References:**

**Abstract #761**

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