NaOH-etched PCLTA and mPEGA/PCLTA Networks for Regulating Smooth Muscle Cells

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Statement of Purpose: The surface physicochemical properties and geometrical features of the underlying biomaterial substrates affect cells through integrins, focal adhesions (FAs), and various pathways.[1] In this study, we photo-crosslinked poly(ε-caprolactone) triacrylate (PCLTA) with methoxy PEG acrylate (mPEGA) to prepare PCLTA networks tethered with PEG chains, in comparison with PCLTA networks. Both PCLTA and mPEGA/PCLTA networks were immersed in 1 M NaOH solution to degrade for different time. The surface properties of the substrates, such as roughness, hydrophilicity, and chemical composition, changed gradually in degradation. The substrates before and after NaOH treatment were used to demonstrate the roles of surface physicochemical and topographical characteristics in regulating primary rat aortic smooth muscle cell (SMC) attachment, proliferation, and alignment.

Methods: PCLTA and mPEGA were synthesized using the methods reported by our research group previously.[2,3] PCLTA with a molecular weight of 11350 g/mol, and mPEGA with a molecular weight of 320 g/mol were chosen to prepare crosslinked substrates. PCLTA(or 5 wt %-mPEGA/PCLTA) /BAPO/CH_2Cl_2 solution (1.5 g/15mg/500μL) was crosslinked under UV light for 20 min (Fig. 1a). Flat crosslinked polymer substrates were soaked in acetone for two days to remove the sol fraction, dried in vacuum, and compressed between two glass plates to smoothen them. Then the substrates were immersed in 1 M NaOH solution to degrade for different time of 0, 24, 48, and 60 h. After being washed 10 times with distilled water, polymer substrates were further soaked in 1 L distilled water for 2 days to fully remove the residual NaOH, and then dried in vacuum. Primary rat aortic SMCs were cultured on these degraded PCLTA and mPEGA/PCLTA substrates for 4 days and characterized.

Results: PCLTA networks were hydrolyzed and degraded gradually in NaOH solution, and thus their surface properties changed. With increasing the hydrolysis time, the water contact angles for both crosslinked PCLTA and mPEGA/PCLTA substrates decreased significantly, and surfaces also became rougher (Fig. 1b). Compared with crosslinked PCLTA substrates, crosslinked mPEGA/PCLTA substrates had lower water contact angles and higher roughnesses at all time points. After the treatment, the decrease in the water contact angle and increase in surface roughness were more obviously for crosslinked mPEGA/PCLTA substrates, suggesting that introduction of mPEGA increased the degradation rate of the PCLTA network. Crosslinked mPEGA/PCLTA substrates supported SMC attachment, proliferation, and spreading better than crosslinked PCLTA, and the cells had smaller circularities (Fig. 1c). These trends remained the same at all the treatment time. Longer NaOH treatment made the substrates support SMC attachment and proliferation better (Fig. 1d). After being treated in NaOH for longer than 48 h, the difference became insignificant. The increases in SMC attachment and proliferation were due to the increases in hydrophilicity and roughness after the degradation. When the substrates were treated for longer time, SMCs on them were larger with smaller circularities. Similar to SMC attachment, the difference was no longer significant between 48 and 60 h for the treatment time. The average area of SMC nuclei was greater on the treated substrates, but no significant differences were found among all the treatment time of 24, 48, and 60 h.

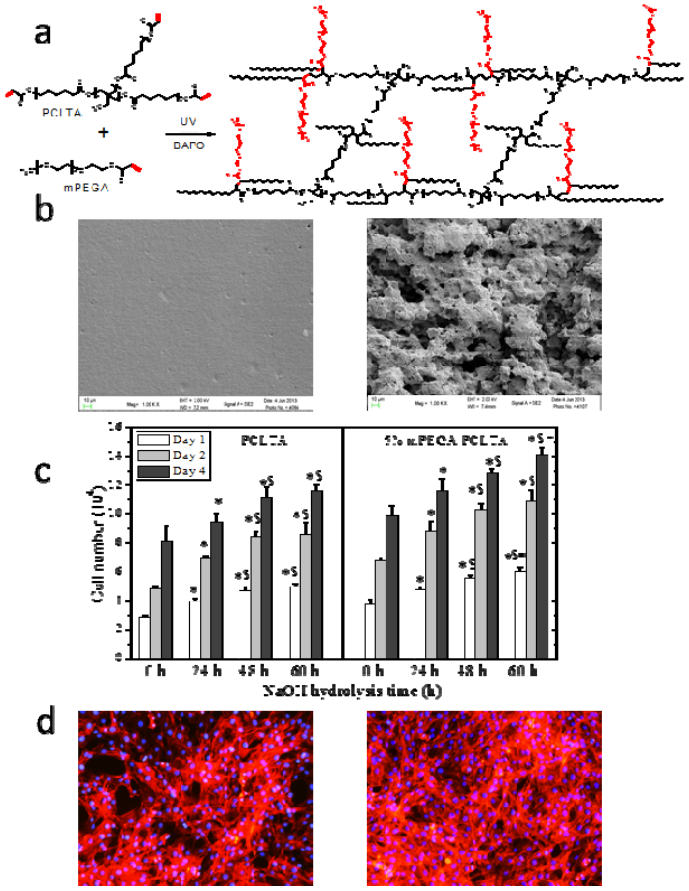

Figure 1. (a) Photo-crosslinking of PCLTA and mPEGA. (b) SEM images of mPEGA/PCLTA substrates before and after NaOH treatment for 60 h. (c) SMC numbers at days 1, 2, and 4. *: $p < 0.05$ relative to 0 h sample; $: $p < 0.05$ to 24 h sample; #: $p < 0.05$ to 48 h sample. (d) Day 4 images of SMCs on crosslinked mPEGA/PCLTA substrates before (left) and after (right) NaOH treatment for 60 h.

Conclusions: Longer NaOH-etching treatment resulted in more hydrophilic and rougher substrates for both crosslinked PCLTA and mPEGA/PCLTA substrates, which better supported SMC attachment, proliferation, spreading, and alignment.

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References: 1. Ingber DE. *FASEB J* **2006**, *20*, 811-827. 2. Cai L. *Polymer* **2010**, *51*, 164-177. 3. Cai L. *Biomacromolecules* **2012**, *12*, 358-368.