Smooth Muscle Cells on Photo-cured Poly(ε-caprolactone) Substrates with Varied Stiffness and Micro-grooves

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Statement of Purpose: The advantages of using biodegradable and photo-crosslinkable polymers in tissue engineering applications are their injectability and feasibility in many fabrication methods such as stereolithography, through which surface micro-patterns can be readily and precisely fabricated.[1-3] In this study, we synthesized four-arm and six-arm poly(ε-caprolactone) acrylates (PCLAs) with different molecular weights. These PCLAs were photo-crosslinked into PCL networks with distinct thermal, rheological, and mechanical properties at physiological temperature. We chose two PCLAs with the highest molecular weights to prepare micro-grooved substrates with groove widths of 5 or 15 μm and groove depths of 1 or 12 μm through replica molding from micro-fabricated silicon chips. Both flat and micro-grooved substrates made from PCLTAs with different molecular weights were used to demonstrate the roles of surface stiffness and microgroove dimensions in influencing smooth muscle cell (SMC) attachment, proliferation, alignment, and gene expression.

Methods: Four-arm/six-arm PCLs with hydroxyl end groups were synthesized via ring-opening polymerization of ε-caprolactone at 130 °C for 12 h in the presence of $Sn(Oct)_2$ as the catalyst and pentaerythritol/ dipentaerythritol as the initiator (Fig. 1a). The molecular weight was modulated using the monomer to initiator ratio. Then acrylation of the 4/6-arm PCL-OH precursors was performed using our previously reported method to obtain PCLAs.[1] PCLA/BAPO/CH_2Cl_2 solution (1.5 g/15 mg/500 μL) was crosslinked under UV light for 20 min. Flat crosslinked PCLA 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. Micro-grooved substrates were prepared by casting PCLA/BAPO/CH_2Cl_2 solution onto silicon molds and photo-crosslinking. Primary rat aortic SMCs were cultured on the flat and micro-grooved substrates for 4 days and characterized.

Results: With more reactive arms than those in PCL triacrylates,[1-3] PCLA networks here were more robust. The stiffness of crosslinked PCLAs can be controlled through the molecular weight, which determines both the crosslinking density and crystallinity.[1-3] At a low molecular weight, PCLA networks were amorphous, and the modulus were inversely proportional to the molecular weight between two neighboring crosslinks. When the molecular weight increased, the networks became semi-crystalline to strengthen the chemical network. Stiffer crosslinked PCLA substrates supported attachment and proliferation of SMCs better (Fig. 1b). No significant differences were observed in SMC numbers at all-time points between micro-grooved and flat substrates, and among different micro-grooved substrates made from the same polymer, except that those with the larger depth of 12 μm and the smaller width of 5 μm (Fig. 1c) had lower cell numbers at days 2 and 4. This reduced cell proliferation was due to the entrapment of cytoplasm and inhibition of spreading in deep and narrow grooves. With increasing the groove depth and decreasing the width, SMCs were smaller. The cell shape was dramatically altered by the groove width and depth. Narrower or deeper grooves induced stronger cell alignment (Fig. 1d). Microgroove dimensions also affected both shape and distribution of SMC nuclei. Cell nuclei were aligned and elongated most in the deeper (12 μm) and narrower (5 μm) microgrooves.

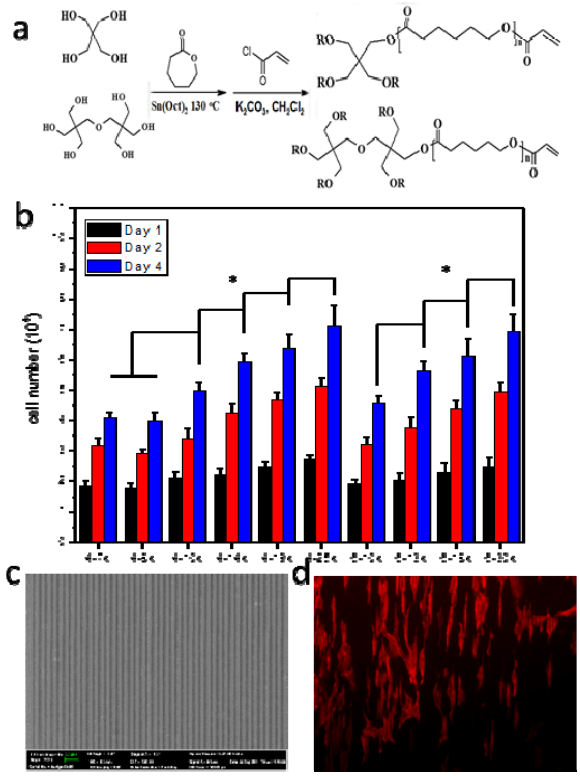

Figure 1. (a) Synthesis of 4-arm/6-arm PCLAs. (b) SMC numbers on flat crosslinked PCLA substrates at days 1, 2, and 4. *: $p < 0.05$. (c) SEM image of a representative micro-grooved substrate with the groove width of 5 μm and the depth of 12 μm. (d) SMCs on the substrate in (c) at day 1, stained with rhodamine-phalloidin.

Conclusions: Stiffer crosslinked PCLA substrates supported SMC attachment and proliferation better. Micro-grooves affected more on alignment and distribution of SMC cytoplasm and nuclei, especially when they were narrower and deeper.

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References: 1. Cai L. *Polymer* **2010**, *51(1)*, 164-177. 2. Wang K. *Adv Healthcare Mater* **2012**, *1*, 292-301. 3. Cai L. *Langmuir* **2012**, *28(34)*, 12557-12568.