Biodegradable Elastomeric Substrates with Concentric Microgrooves for Regulating MC3T3 Cell Behavior

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Statement of Purpose: Injectable and *in situ* photo-crosslinkable poly(ε-caprolactone) triacrylates (PCLTAs) recently developed in our group are suitable for bone regeneration because of efficient curing, excellent biocompatibility, and controllability in mechanical properties[1]. Micro- and nano-structured surface features have demonstrated regulation of cell/substrate interactions on various materials[2]. Two distinct PCLTAs have been used to fabricate concentric microgrooves with varied width and depth to investigate mouse MC3T3-E1 cell behavior. Preference to the stiffer substrate of crosslinked PCLTA has been demonstrated in cell proliferation, alignment, elongation, nucleus distribution, and mineralization. Microgrooves with a narrow width of 7.5 μm and a depth of 10 μm could align and elongate cytoskeleton and nuclei most efficiently and more interestingly, induce most significant cell differentiation.

Methods: Two PCLTAs (Figure 1a) were synthesized via first ring-opening polymerization of ε-caprolactone initiated by 1,1,1-tris(hydroxymethyl) propane and then acrylation with acryloyl chloride in the presence of K_2CO_3[1]. PCLTA10k and PCLTA7k had M_n of 9750, 6680 g.mol^{-1} and M_w of 12310, 8490 g.mol^{-1}, respectively. PCLTA was dissolved in methylene chloride and photo-crosslinked on silicon micro-machined template wafers with concentric structures and peeled off.

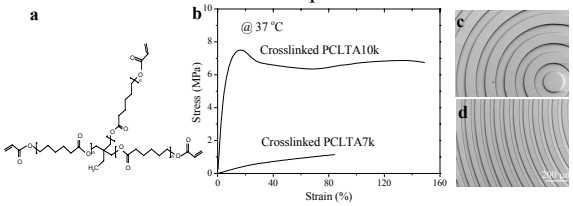

Figure 1. (a) Chemical structure of PCLTA. (b) Stress-strain curves for two PCLTA networks at 37 °C. SEM images of crosslinked PCLTA7k microgrooves with widths of (c) 90 μm and (d) 45 μm and a depth of 10 μm.

Results: Concentric microgrooves with four widths of 7.5, 15, 45, and 90 μm and three depths of 0.2, 1, and 10 μm have been fabricated successfully using two PCLTAs (Figure 1c,d). Semi-crystalline crosslinked PCLTA10k demonstrated stronger mechanical properties with a tensile modulus of 105.9 ± 23.6 MPa, two orders of magnitude higher than the value of 1.61 ± 0.46 MPa for amorphous crosslinked PCLTA7k (Figure 1b). MC3T3 cells could proliferate faster on the stiffer substrate although no differences were found when feature dimensions were varied on the same material. Cell elongation and alignment was more significant on narrower grooves and stiffer crosslinked PCLTA10k after attachment (Figure 2a,b). Cell nuclei were also elongated better on narrower microgrooves and along the edges of wider microgrooves (Figure 2c). Because of better cell alignment and communication, significantly better MC3T3 cell differentiation was found on the stiffer substrates and the narrower grooves, demonstrated by the calcium content stained using Alizarin red S in Figure 2d,e. Distinct cell behavior was further illustrated by MC3T3 cell images (Figure 2e).

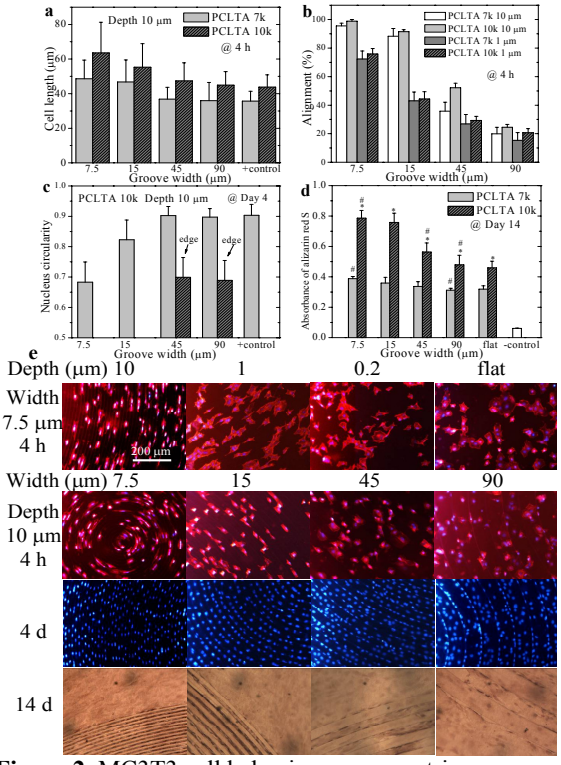

Figure 2. MC3T3 cell behavior on concentric microgrooves with different dimensions and two polymers. (a) cell length, (b) percentage of aligned cells at 4 h, (c) nucleus circularity at day 4, (d) calcium content at day 14, (e) cell images at different days with actin filaments (red), nuclei (blue), and Alizarin red S stain (dark red). Scale bar of 200 μm is applicable to all in (e). *, $p < 0.05$ compared to corresponding PCLTA7k. #, $p < 0.05$ between two marked samples.

Conclusions: Using replica molding from silicon wafers, concentric microgrooves with different depths and widths have been fabricated using two biodegradable and photo-crosslinkable PCLTAs that could result in distinct mechanical properties. Both intrinsic material properties and surface features demonstrated strong influence on MC3T3 cell behavior. Cell alignment and elongation, nuclei distribution, and mineralization have been found to be more significant on stiffer crosslinked PCLTA10k and narrower microgrooves with a width of 7.5 μm and a depth of 10 μm. The results offer guidance for fabricating 3D scaffolds to promote bone repair and regeneration.

References: 1. Cai, L. *Polymer* **2010**, *51*, 164.
2. Bettinger, C. J. *Angew Chem Int Ed Engl Ed* **2009**, *48*, 5406.