Biodegradable Elastomeric Substrates with Micro-fabricated Grooves for Promoting Neurite Extension

Lei Cai,[a] Li Zhang,[b] Jingyan Dong,[b] and Shanfeng Wang[a]

[a] Department of Materials Science and Engineering, The University of Tennessee, Knoxville, TN 37996; [b] Edward P. Fitts Department of Industrial and Systems Engineering, North Carolina State University, Raleigh, NC 27695

Statement of Purpose: Photo-crosslinkable and biodegradable nerve conduits can be used to guide axonal growth in peripheral nerve regeneration[1]. Although micro- and nano-structured surfaces have been widely examined in regulating cell behavior, not many biodegradable elastomers have been developed for guiding nerve cells[2]. In this study, two poly(ε-caprolactone) triacrylate (PCLTA) have been photo-crosslinked into elastomeric substrates with parallel microgrooves of different widths and depths. We further investigated rat Schwann cell precursor line (SpL201) and pheochromocytoma (PC12) cell behavior on these substrates. Distinct preference to the substrates with different mechanical properties has been demonstrated for these two cell types. Cytoskeleton and nuclei can be altered by varying the microgroove depth and width. Neurites can be oriented as well with better extension on narrower and shallower microgrooves.

Methods: PCLTA was synthesized using ring-opening polymerization of ε-caprolactone using 1,1,1-tris(hydroxymethyl) propane as the initiator, followed by acrylation with acryloyl chloride in the presence of K_2CO_3[3]. PCLTA10k and PCLTA7k had M_n of 9750, 6680 g.mol^{-1} and M_w of 12310, 8490 g.mol^{-1}, respectively. Polymer solutions in CH_2Cl_2 were photo-crosslinked on silicon molds with parallel microgrooves.

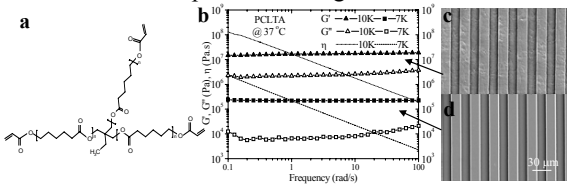

Figure 1. (a) Chemical structure of PCLTA. (b) Storage modulus G', loss modulus G'', and viscosity η vs. frequency for PCLTA networks at 37 °C. SEM images of crosslinked PCLTA10k (c) and 7k (d) substrates with microgrooves (15 μm width, 5 μm depth).

Results: Elastomeric substrates fabricated using PCLTA7k and 10k had sufficiently high gel fractions for achieving parallel microgrooves with integrity and various widths of 5, 15, 45, and 90 μm and depths of 0.4, 1, 5, and 12 μm. Enhanced by crystalline domains, the tensile and shear moduli of crosslinked PCLTA10k were two orders of magnitude higher than those of amorphous crosslinked PCLTA7k (Figure 1b-d). Stiffer substrate of crosslinked PCLTA10k better supported proliferation of glial-natured SpL201 cells but did not promote neuronal-like PC12 cell proliferation, although no difference was found when the microgroove dimension was varied. Nuclei of both cell types were found to be confined in the microgrooves with a narrow width of 5 μm (Figure 2a). Deeper grooves of 12 μm could further elongate nuclei (Figure 2b). PC12 neurites were also aligned along the narrow grooves of 5 or 15 μm and the edge of wide grooves by showing small angles between grooves and neurites (Figure 2c). Oriented but not fully confined neurites were found to be the longest on the narrowest and shallowest microgrooves (Figure 2d). Cell images in Figure 2e further demonstrated the distinct cell numbers, elongation and alignment, and neurite extensions on these two substrates with different microgroove dimensions.

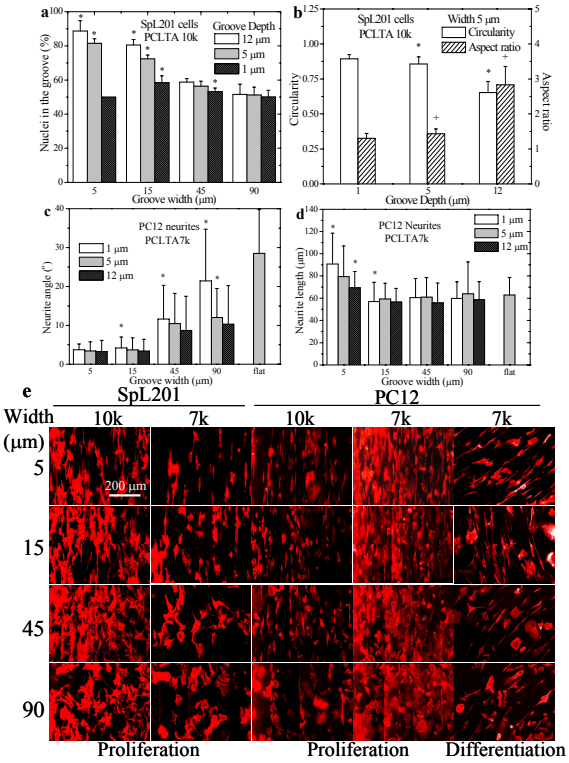

Figure 2. (a) Percentages and (b) circularity and aspect ratios of SpL201 nuclei in the microgrooves of crosslinked PCLTA10k at day 7 post-seeding. (c) PC12 neurite angles and (d) lengths on crosslinked PCLTA7k with different microgrooves. (e) SpL201 and PC12 cell images on microgrooves with a depth of 5 μm and PC12 differentiation on microgrooves with a depth of 0.4 μm at day 7. Scale bar of 200 μm is applicable to all in (e). *, $^{+}$: $p < 0.05$ between two marked samples.

Conclusions: Parallel microgrooves with different depths and widths have been fabricated using two biodegradable and photo-crosslinkable PCLTAs. Both intrinsic material properties and feature dimensions influenced SpL201 and PC12 cell behavior as more significant cell elongation and alignment, nuclear polarization, and oriented neurite extension were found on narrower microgrooves.

References:

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3. Cai, L. *Polymer* **2010**, *51*, 164.

Abstract #543