Poly(ethylene glycol)-tethered Biodegradable Elastomers for Regulating Surface Characteristics and Nerve Cell Behavior

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Statement of Purpose: Injectable and photo-crosslinkable poly(ε-caprolactone) diacrylate (PCLDA) has been synthesized in our group for diverse tissue-engineering applications[1]. After crosslinking, PCLDA networks have excellent cytocompatibility and controllable mechanical properties by varying the crosslinking density and crystallinity[1]. Semi-crystalline PCLDA networks were demonstrated to be supportive for mouse MC3T3 cells and rat Schwann cell precursor line (SpL201) cells but their performance was limited by surface hydrophobicity. In this study, PCLDA networks were modified by incorporating hydrophilic methoxy poly(ethylene glycol) monoacrylate (mPEGA) at various compositions (ϕ_{mPEGA}) from 0 to 30%. Improved wettability has been achieved with a corresponding decrease in protein adsorption as the result of repellent PEG chains tethered in the network. SpL201 and pheochromocytoma (PC12) cells have been used to evaluate their responses to the modified surfaces. Non-linear or parabolic dependence of cell proliferation and differentiation on ϕ_{mPEGA} has been found for both cell types with maximal values at ϕ_{mPEGA} of 5-7%.

Methods: Methoxy polyethylene glycol (mPEG) with nominal molecular weight of 350 $g.mol^{-1}$ from Sigma was used to synthesize mPEGA by reacting with acryloyl chloride in the presence of K_2CO_3[2]. mPEGA and PCLDA (M_n = 3510 $g.mol^{-1}$, M_w = 5150 $g.mol^{-1}$) were dissolved in CH_2Cl_2 and photo-crosslinked (Figure 1).

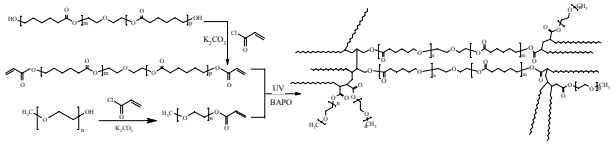

Figure 1. Synthesis and crosslinking of mPEGA/PCLDA.

Results: Both water contact angle and the ability of adsorbing serum proteins from culture medium decreased progressively with increasing ϕ_{mPEGA}, originated from the hydrophilic nature and repulsive effect of PEG chains, respectively (Figure 2a). SpL201 cell proliferation demonstrated a parabolic trend with ϕ_{mPEGA} and maximized at ϕ_{mPEGA} of 5-7% (Figure 2b). The improved hydrophilicity at low densities of tethered mPEG chains benefited cell growth while less protein adsorption and the repulsion of the tethered PEG chains dramatically inhibited cell proliferation at higher ϕ_{mPEGA} of 20%-30%. Differentiated SpL201 cells after forskolin treatment were found to have the largest O4-positive areas on the substrate with intermediate hydrophilicity (Figure 2c), indicating these surfaces could better upregulate embryonic markers toward cell maturation and myelination[3]. The parabolic trend existed not only for glial-natured SpL201 cells, but also for proliferation and differentiation of PC12 cells, a neuronal-like model cell line that can be induced by nerve growth factor to extend neurites. The number of neurites per cell, percentage of cells bearing neurites, and neurite lengths all showed significantly higher values on the substrates with ϕ_{mPEGA} of 5% than crosslinked PCLDA (Figure 2d). Cell images and phenotype (Figure 2e) were consistent with the cell numbers measured using fluorescence-based assay.

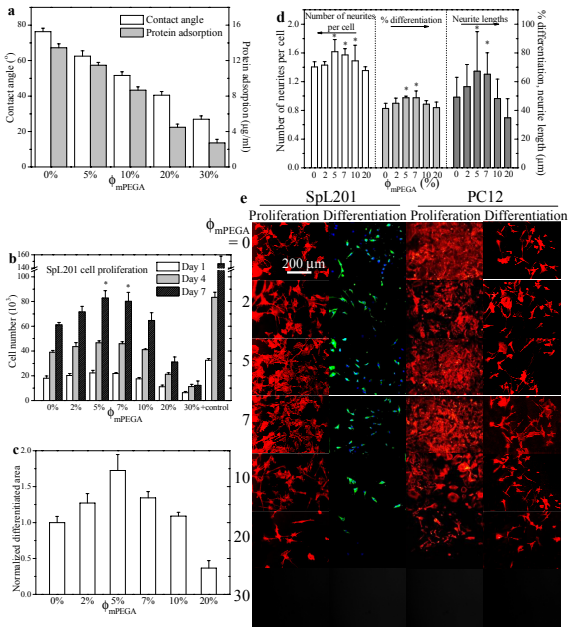

Figure 2. (a) Contact angle and protein adsorption, (b) SpL201 cell proliferation at days 1, 4 and 7, (c) SpL201 cell differentiation quantified using areas positive to O4 marker, (d) PC12 neurite extension, and (e) cell images for actin filaments (red), nuclei (blue) or O4-positive areas (green) at day 7 on the crosslinked PCLDA and mPEDA/PCLDA blends. Scale bar of 200 μm is applicable to all in (e). *, $p < 0.05$ compared to other samples in (b). $p < 0.05$ between two neighboring samples in (c).

Conclusions: mPEGA end-capped with one double bond has been photo-crosslinked with semi-crystalline PCLDA to modify its surface chemistry. Improved hydrophilicity and reduced protein adsorption played collective roles to regulate SpL201 and PC12 cell proliferation and differentiation. Proliferation of both cell types were first promoted by moderate hydrophilicity at ϕ_{mPEGA} of 5-7% and then suppressed on surfaces tethered with more PEG chains. The same trend was found in SpL201 and PC12 cell differentiation. The present method represents an efficient means to tune the surface chemistry for enhancing regenerative functions of nerve cells while inhibiting fibrous tissue growth during nerve regeneration.

References: 1. Cai, L. *Polymer* **2010**, *51*, 164.
2. Cai, L. *Biomaterials* **2010**, *31*, 4457.
3. Lobsiger, C. S. *Glia* **2001**, *36*, 31.