**Regulation of Smooth Muscle Cell Behavior on Novel Biodegradable Elastomeric Substrates with Controllable Stiffness**

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**Statement of Purpose:** Smooth muscle cells (SMCs) exhibit phenotype plasticity that is of critical importance for blood vessel regeneration because synthetic phenotype is needed for cell proliferation and tissue remodeling while contractile phenotype is necessary for forming functional blood vessel. It has been found that substrate stiffness could influence SMC phenotype remodeling and lead to modulation of gene expression[1-2]. Challenge lies in designing biomaterials that can support fast proliferation of synthetic SMCs and also facilitate the phenotypic conversion into functional contractile SMCs. Photo-crosslinkable poly(ε-caprolactone) triacrylates (PCLTAs) recently developed in our group have controllable physical properties after crosslinking for diverse tissue-engineering applications[3]. In this study, we have investigated phenotypic modulation of SMCs on elastomeric substrates fabricated by PCLTAs with six molecular weights ranging from 2000 to 20000 g/mol. Through controlling the crosslinking density and crystallinity of the networks, these substrates demonstrated parabolic dependent stiffness on the molecular weight. We further analyzed the relative gene expression of synthetic marker non-muscle myosin heavy chain (NM-MHC) and contractile markers including smooth muscle myosin heavy chain (SM-MHC), transgelin, smoothlin, and calponin, normalized using the house-keeping gene GAPDH.

**Methods:** To synthesize PCLTAs, PCL triol, acryloyl chloride, and $K_2CO_3$ were reacting under nitrogen for 24 hours in a molar ratio of 1:3:3 at room temperature. PCLTA networks were formed via photo-crosslinking as described previously[3]. Primary SMCs isolated from rat aorta were cultured on six photo-crosslinked PCLTA sheets at a seeding density of ~15,000 cells/cm$^2$ for 3 days. After cDNA was obtained using reverse transcription polymerase chain reaction (RT-PCR) of RNA, the relative expressions of one synthetic marker and four contractile markers were determined on both RT-PCR and SYBR® green real-time PCR.

**Results:** Six photo-crosslinked PCLTA networks demonstrated controllable mechanical properties by tuning the crosslinking density and crystallinity simultaneously. As shown in Figure 1a, tensile modulus exhibited a parabolic trend as the molecular weight of PCLTA increased. The initial decrease in tensile modulus was the results of decreasing crosslinking density while the later increase originated from enhancement of crystalline domains. Normalized SMC attachment at 4 h was significantly more on stiffer semi-crystalline networks (Figure 1c), on which cells were more spread-out with larger areas (Figure 1d). Individual cell images at day 1 stained using rhodamin-phalloidin demonstrated more obvious actin filaments on stiffer substrates (Figure 1e). SMC proliferation at day 1, 2 and 4 post-seeding showed an evident decreasing trend from crosslinked PCLTA2k to 7k and an increasing trend from crosslinked PCLTA7k to 20k, coincident with the trend in tensile modulus (Figure 1b). RT-PCR and Real-time PCR results were consistent with each other and both demonstrated that semi-crystalline PCLTA networks, which had higher tensile moduli than those of amorphous PCLTA networks, could better support gene expression of SMC contractile phenotypic marker (Figure 1f-i).

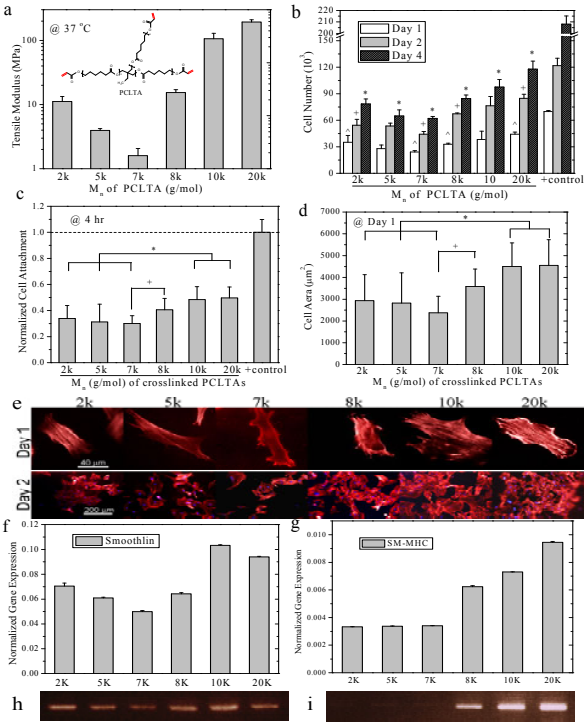


Figure 1. (a) Tensile moduli of crosslinked PCLTAs at 37 °C. (b) SMC number at days 1, 2, and 4 post-seeding. ^, $^+$, *: $p < 0.05$ between any two samples marked with the same symbol. (c) Normalized SMC cell attachment at 4 h post-seeding. (d) Average cell projection area at day 1 on crosslinked PCLTA disks. $^+$, *: $p < 0.05$ between two marked samples. (e) Cell images stained using rhodamin -phalloidin (red) and 4',6-diamidino-2-phenylindole (DAPI, blue). Real time PCR results of smoothlin (f) and SM-MHC (g). RT-PCR results of smoothlin (h) and SM-MHC (i).

**Conclusions:** Photo-crosslinked PCLTAs with controllable stiffness have been achieved by varying the crosslinking density and crystallinity simultaneously. Distinct smooth muscle cell responses have been found to these substrates. Semi-crystalline PCLTA networks having higher tensile moduli than those of amorphous PCLTAs could better support SMC attachment, spreading, proliferation, and contractile gene expression.

**References:** 1. Discher DE. *Science* **2005**, *310*, 1139-43.
2. Haga JH. *Journal of Biomechanics* **2007**, *40*, 947-960
3. Cai L. *Polymer* **2010**, *51*, 164-177