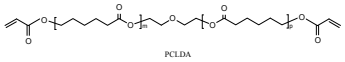
Poly(ε-caprolactone) Acrylate Networks with Controllable Properties and Tunable Cell Behavior

Lei Cai and Shanfeng Wang

Department of Materials Science and Engineering, The University of Tennessee, Knoxville, TN 37996

Statement of Purpose: Based on poly(ε-caprolactone) (PCL) diol, numerous photo-crosslinkable copolymers have been synthesized through condensation with small molecules such as acryloyl chloride and fumaryl chloride or macromers such as poly(propylene fumarate) (PPF).[1-3] Specifically, PCL diacrylates (PCLDAs) have been synthesized in benzene in the presence of a proton scavenger, triethylamine (TEA).[1] We present a facile method to synthesize photo-crosslinkable poly(ε-caprolactone) acrylates (PCLAs) with controllable physical properties to satisfy diverse tissue-engineering needs such as bone and nerve regeneration. This novel method uses K_2CO_3 as the proton scavenger to avoid side reactions and to simplify purification. Through combining a crystallite-based physical network and a crosslink-based chemical network together, we could modulate material properties and consequently control cell responses. Polymer disks, tubes, and porous scaffolds have been fabricated via photo-crosslinking. Thermal, mechanical, rheological, and surface properties have been characterized. Mouse MC3T3 cells and rat Schwann precursor cell line (SPL201) cells have been applied to evaluate the *in vitro* biocompatibility and the roles of surface chemistry, crystallinity, and stiffness in regulating cell attachment, spreading and proliferation collectively.

Methods: PCLAs were synthesized end-capping PCL diols (nominal M_ns of 530, 1250, 2000 g/mol) and triols (nominal M_ns of 300 and 900 g/mol) with acryloyl chloride in anhydrous methylene chloride. The chemical structure of PCLDA is shown in Figure 1. Excessive K_2CO_3 were used as a proton scavenger. Polymer disks, strips, nerve tubes and porous scaffolds were fabricated using the photo-crosslinking method described previously.[4] The details about material characterizations and cell studies can be found in earlier reports.[4]



PCLDA

Figure 1. Chemical structure of PCLDA.

Results: PCLA networks could be efficiently fabricated via photo-crosslinking with gel fractions higher than the values of photo-crosslinked PCLFs.[4] Crosslinked PCLDA530, PCLTA300 and 900 were completely amorphous without the enhancement of crystallites. Thus, their mechanical properties were determined by crosslinking density. The moduli of crosslinked PCLDA530 were the lowest among PCLDAs and the moduli of crosslinked PCLTA300 were higher than those of PCLTA900. PCLDA1250 and 2000 were semi-crystalline with a crystallinity of 30.4% and 34.1%, respectively. With crystallites serving as physical fillers and forming a physical network, crosslinked PCLDA2000 with the highest crystallinity and T_m among these samples had the highest tensile, shear, compression, and torsional moduli at 37 °C. All crosslinked PCLAs demonstrated no crytotoxicity in 4 and 7 days for MC3T3 and SPL 201 cells, respectively. Cell attachment is illustrated in Figure 2B, suggesting that different cell types responded to one substrate differently with the same preference for PCLDA2000. Cell images in Figure 2A demonstrated the spread-out phenotype of MC3T3 cells and they were consistent with the cell numbers shown in Figure 2C. Substantial cell proliferation could be found on the disks of crosslinked PCLDA2000 while it was weaker on the disks of crosslinked PCLDA1250 and 530. Similar trend was also found in SPL201 cells.

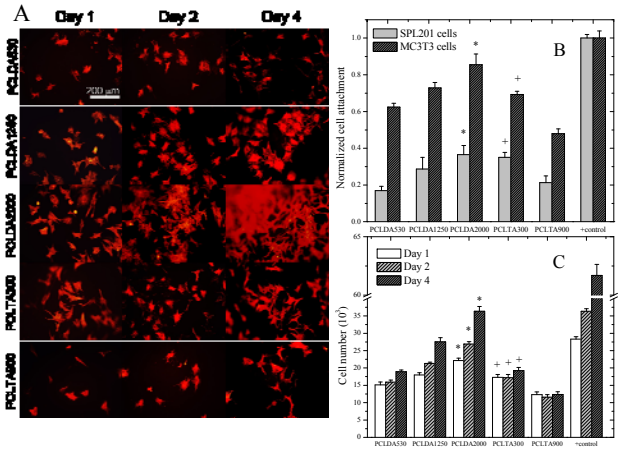


Figure 2. A: Morphology of mouse MC3T3 cells on the crosslinked PCLA disks at day 1, 2, and 4 post seeding. The scale bar of 200 μm is applicable to all. B: Normalized MC3T3 and SPL201 cell attachment at 4 hr. C: MC3T3 cell proliferation in 4 days post seeding, compared to cell-seeded TCPS as positive control. *, $p<0.05$ relative to PCLDA530, PCLDA1250 and TCPS. +, $p<0.05$ relative to PCLTA900 and TCPS.

Conclusions: A facile method has been applied to synthesize a series of crosslinkable PCLAs in the presence of K_2CO_3 instead of TEA. Light-colored PCLAs are more efficient and convenient for photo-crosslinking and cell studies. Among five PCLAs, photo-crosslinked PCLDA2000 with the highest crystallinity and mechanical properties was the most favorable material for cell attachment, spreading, and proliferation tested using both mouse MC3T3 and rat SPL 201 cells. This phenomenon can be tentatively attributed to crystallinity-enhanced surface stiffness. Together with excellent cytocompatibility, these polymers are able to form disks, tubes, and scaffolds, demonstrating their potentials as injectable biomaterials for diverse tissue engineering applications.

References:

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