Photo-crosslinked Polymer Nanowires for Regulating Smooth Muscle Cells

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Statement of Purpose: Inorganic nanoporous aluminum oxide (AAO) templates were used to fabricate photo-crosslinked poly(ε-caprolactone) triacrylate (PCLTA) nanowire substrates with three different wire diameters of 20, 100, and 200 nm. The length and morphology of these nanowires were well controlled by adjusting the PCLTA solution concentration and the pore size of the AAO templates. Photo-crosslinked PCLTA nanowires were characterized in terms of surface morphology, hydrophilicity, and serum protein adsorption. Smooth muscle cells (SMCs) cultured on these polymer nanowire substrates were used to study their attachment, proliferation, spreading, differentiation, and cellular sensing components as focal adhesions and integrins.

Methods: Ring-opening polymerization of ε-caprolactone into PCL triol was initiated by 1,1,1-tris(hydroxymethyl) propane with $Sn(Oct)_2$ as the catalyst. PCL triol was further acrylated into PCLTA in the presence of potassium carbonate [1,2]. Phenyl bis(2,4,6-trimethyl benzoyl) phosphine oxide (BAPO, IRGACURE819, Ciba Specialty Chemicals, NY) was used as the initiator in photo-crosslinking PCLTA. As shown in Fig. 1a, a layer (~0.1 mm) of PCLTA/BAPO/ CH_2Cl_2 mixture was placed onto glass slides (25.4×76.2×1.0 mm, width × length × thickness). Three types of inorganic AAO templates (Anodisc, Whatman) with different pore diameters of 200, 100, and 20 nm were placed onto the polymer layer. Then the polymer/template pairs were placed under a UV lamp (SB-100P, Spectroline, wavelength = 365 nm, intensity = 4800 μw/cm^2) for 30 min to photo-crosslink PCLTA. After crosslinking, the polymer/template pairs were immersed in 1 M NaOH solution with gentle stir for 40 min to dissolve the AAO templates [3]. In cell studies, primary SMCs isolated from rat aorta were cultured in DMEM containing 10% fetal bovine serum on the photo-crosslinked PCLTA nanowire substrates at a density of ~15,000 cells/cm^2.

Results: The fabrication method using inorganic AAO templates was successful for making photo-crosslinked PCLTA nanowire substrates, which were characterized using SEM images in Fig. 1b. On nanowire substrates, the water contact angles were higher than on the flat one made from the same photo-crosslinked PCLTA, indicating that the nanowire substrates were more hydrophobic. As shown in Fig. 1c, SMCs on flat crosslinked PCLTA had a small spread area and regular distribution of focal adhesions. In contrast, confocal microscopic images of SMCs on the nanowire substrates showed significantly better spreading and stronger focal adhesions. We found better SMC attachment at 4 h post-seeding and faster SMC proliferation over 4 days on the nanowire substrates than on the flat one (Fig. 1d). These results indicated that the nanowire structures fabricated from photo-crosslinked PCLTA could promote better SMC attachment, spreading, proliferation, and focal adhesion development.

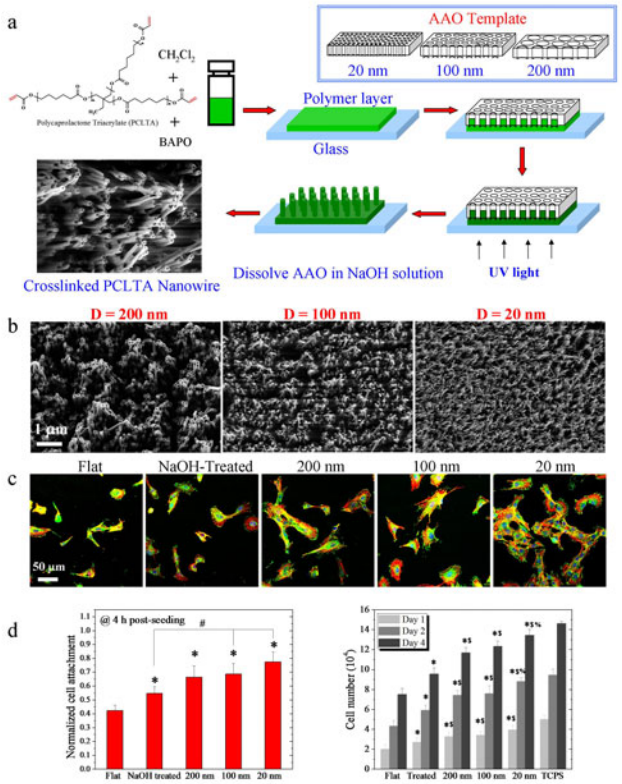

Figure 1. (a) Schematic fabrication of photo-crosslinked PCLTA nanowire substrates using AAO templates with different pore diameters. (b) SEM images of photo-crosslinked PCLTA nanowire substrates with three different nanowire diameters. (c) Confocal microscopic images of SMC focal adhesions on the photo-crosslinked PCLTA nanowire substrates. (d) SMC attachment and proliferation on these nanowire substrates.

Conclusions: Photo-crosslinked PCLTA nanowire substrates were successfully fabricated using nanoporous AAO templates. Compared with the flat one, these photo-crosslinked polymer nanowire substrates adsorbed more serum proteins from the culture media for SMCs, became more hydrophobic, enhanced SMC attachment, proliferation, spreading, and differentiation, especially when the nanowires were smaller. Further analysis of cellular sensing components indicated that small-diameter nanowires triggered stronger focal adhesions and higher expression levels of integrin subunits. Cellular interactions with photo-crosslinked PCLTA nanowire substrates are useful for advancing their cardiovascular tissue engineering applications.

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References: 1. Cai L. *Polymer* **2010**, *51*, 164-177.
2. Liu X. 242nd *ACS National Meeting*, Polymer Division **2011**,*105*, 124-126.
3. Du K. *ACS Appl Mater Interfaces* **2012**, *4(9)*, 4643-4650.