The role of surface topography on cellular attachment to poly(L lactic acid) electrospun fibers

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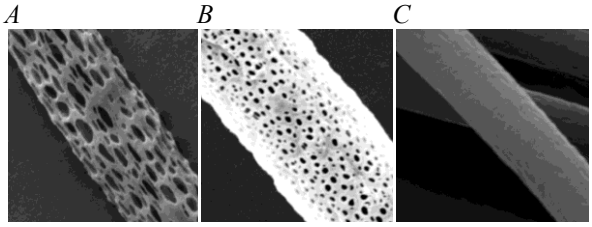
Statement of Purpose: Electrospinning has been shown to be a promising technique for the fabrication of novel tissue scaffolds for tissue engineering. Topographical patterning of biomaterial surfaces can have significant effects on cell attachment, proliferation and differentiation[1]. Polymers electrospun from certain solvents in controlled environments have been shown to form pores on the surface. The pores may be dependent on many factors including the presence or absence of humidity and the molecular weight of the polymer[2]. By adjusting the molecular weight, we can create different nano-morphologies to study the cell interactions with the poly(L-lactic acid) (PLLA) surface. Two different molecular weight samples of PLLA were electrospun under similar conditions and cultured for 3 days with L929 fibroblasts. By increasing/decreasing the size of nanopores on the surface of electrospun fibers, the attachment behavior of L929 fibroblast cells (ATCC) can be altered.

Methods: Two different PLLA samples (high and low molecular weight PLLA, respectively) were obtained and used as received. All samples were electrospun under conditions determined to be required for the production of bead-free fibers with varying levels of porosity. Nonwoven fiber mats were mounted on SEM stubs, coated with Au/Pd (Quorun Technology) and imaged with a Zeiss Evo LS15 scanning electron microscope. Pore characterization was done using ImageJ (NIH) software.

Cell and Sample Preparation: Sterile samples (n=4 per group, 4 groups) were transferred to individual wells of two, twelve-well nontreated culture plates. Complete media (EMEM (ATCC) + 10% FBS (Invitrogen)) was added to each well (1 ml per well) and incubated for one hour under standard conditions (37°C, 5% CO_2) to precondition the samples with protein to enhance cell attachment. After one hour, the media was removed from the wells and 1 ml of media containing 57,500 cells were added to each well and the samples incubated for four hrs to allow for cell attachment, after which, the samples were transferred to new twelve-well plates and 1 ml of standard media was added to each well. Live/Dead (Invitrogen) staining kit was used per manufacturer's instructions. Images were acquired using a Zeiss Axiovert 200M equipped with an AxioCam HDR camera. Twenty images were acquired for each sample group (10-10x and 10-20x images). After imaging the samples, they were transferred to a new twelve well plate and stored in 10% neutral buffered formalin. After the fixation, samples were critical point dried, sputter coated with Au/Pd (Quorun Technology) and imaged with a Zeiss Evo LS15 scanning electron microscope.

Results and Discussion: The average pore size produced from the high molecular weight sample was 475 nm^2 and the average pore size from the low molecular weight samples was 45 nm^2 (Figure 1, Table 1). After three days of culture, the L929 fibroblasts were viable and had attached to electrospun fibers in all test groups (Figure 2). Upon visual inspection, it appears that porous fibers promoted increased cell attachment (Fig. 2 c and d) and that cells cultured on the high molecular weight porous fibers (Fig 2 d) had increased spread area compared to those on the lower molecular weight fibers (Fig. 2 c).

Figure 1



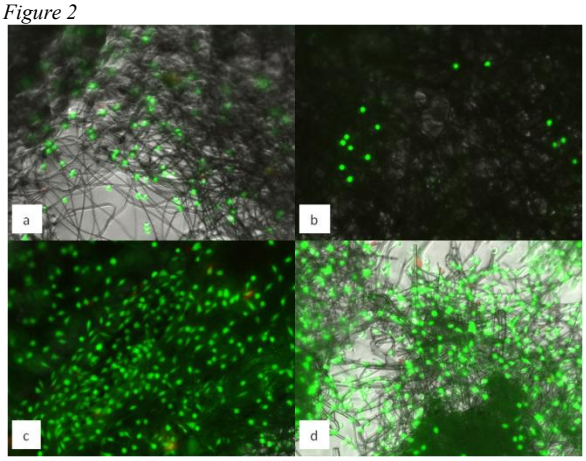
Electrospun poly(L)lactic acid(PLLA) fibers, A) High molecular weight PLLA, B) Low molecular weight PLLA C) Low molecular weight PLLA.

Table 1

MW	*Pore Length*	*Pore Width*	*Pore Area**
High	*634+/-231*	*240+/-116*	*475*
Low	*125+/-27*	*114+/-20*	*45*

*Pore analysis for samples used in cell cultures. *Pores were assumed to be approximately elliptical (n=100).*

Figure 2



Live/Dead staining of (a) high mw non porous, (b) low mw nonporous, (c) high mw porous, (d) low mw porous fibers.

References:

1- Hu, J. Microelect Eng 87 (2010) 726-729
2- Casper, C.LMacromolecules 2004, 37, 573-578
3- Shenoy, S.L. Polymer, 46 (2005) 3372-3384