Brush-On Fabrication of Suspended PLLA-PEO-PLLA Triblock Copolymer Fibers

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ABSTRACT

Parallel fibers can be rapidly created by manually brushing solvated polymer solutions over arrays of microfabricated pillars. Recently, this technique has been utilized to produce suspended arrays of micron and sub-micron scale fibers consisting of a new triblock copolymer composed of a hydrophilic mid-block of polyethylene oxide (PEO) surrounded by two hydrophobic end groups of poly-L-lactic acid (PLLA). A solution of this polymer in chloroform has been used to fabricate fibers with diameters ranging from 125 nm to >50 μm. Annealing these fibers in water induces the formation of ultrastructural nanoscale pores that can be selectively oriented through variation of the surface treatment. Because the PLLA-PEO-PLLA triblock copolymer is both biocompatible and biodegradable, these arrays of fibers are excellent candidates for biomimetic extracellular matrix (ECM) structures. Additionally, the porous ultrastructure of the fibers may provide a platform for the *in situ* delivery of growth factors to stimulate endothelial cell proliferation, migration, and lumen formation.

INTRODUCTION

The selective generation of new blood vessels from existing vessels or precursor cells would be a critically useful tool in tissue engineering (TE). The development of complex microvascular networks is an essential prerequisite for the establishment of larger tissues, which require adjacent capillary networks to provide delivery of nutrients and removal of waste products. The development of a synthetic extracellular matrix (ECM) would alleviate this impediment by mimicking the natural extracellular matrices that initiate and sustain neovascularization. A viable synthetic ECM would enable detailed evaluation of angiogenic phenomena, including studies of neovascularization during wound healing, or where unwanted vascularization occurs, such as with cancerous growth or rheumatoid arthritis [1].

A synthetic ECM must meet several criteria to be viable. First, the ECM must be capable of guiding and supporting cellular growth. Second, the ECM must provide a mechanism for delivering angiogenic growth factors to the cells that encourages the cells to develop into functional capillaries. Third, the synthetic ECM must maintain its dimensions and structural integrity over the timeframe of capillary regeneration. [2, 3].

Scaffolds made from the triblock copolymer poly(L-lactic acid-*b*-ethylene oxide-*b*-L-lactic acid) (PLLA-PEO-PLLA) appear to satisfy these criteria. This amphiphilic polymer consists of a hydrophilic PEO center group positioned between two chains of the hydrophobic and biodegradable polyester, PLLA (Figure 1). Tew *et al.* have demonstrated that, in the gel phase, this polymer can be tailored through chain length alteration to more closely mimic the stiffness of natural tissues when compared with other common cell scaffold materials [4]. Additionally, it has been demonstrated that this hydrogel material can be loaded with bioactive molecules and utilized as a nanoparticle drug delivery system [5-8].
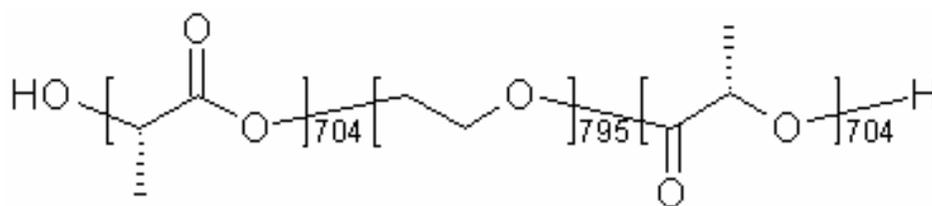
Figure 1 – Chemical structure of PLLA-PEO-PLLA triblock copolymer.

The fabrication and processing of this polymer can be varied to produce suspended fibers of widely varying diameters and morphology. Perhaps the most significant and unresolved factor affecting tissue growth is how surface morphology influences cell shape, adhesion, cell cycle, and growth [9-11]. The processing methods of PLLA-PEO-PLLA reported herein enable substantial variation and control of the properties of cell scaffolds, which are needed to determine the preferred conditions for tissue regeneration.

APPROACH TO FABRICATION OF SCAFFOLDS

Several groups have fabricated and evaluated fibrous scaffolds of biodegradable polymers for cellular support and drug delivery. Fibrous polymer matrices have been made by melt-blowing, phase separation, wet spinning, and dry spinning. However, the most commonly reported method of scaffold fabrication is by electrospinning, in which a strong electric field is utilized to process a solvated polymer into a randomly woven or semi-aligned mat of micro- and/or nanoscale fibers [12-15]. Electrospinning does not permit precise point-to-point positioning of the fibers and the surface is typically smooth and non-porous. The approach we present enables textured, porous and point-to-point positioning.

Our group recently reported that suspended micro- and nanoscale polymer fibers could be formed by manually brushing solvated polymers across microscale structures [16]. A small volume of polymer solution is applied to a glass coverslip applicator (Figure 2a), which is manually brushed across the tips of microfabricated pillars. Solution that adheres to a pillar is drawn into a suspended thread in the direction of brushing, which becomes attached to pillars (Figure 2b). These filaments thin, through surface tension-driven necking (i.e. capillary thinning). As the solvent evaporates the fibers solidify producing suspended, micro- and nanoscale fibers (Figure 2c). To date, the brush-on technique has been utilized to fabricate fibers from a variety of polymers and polymer-based composites including poly(methyl methacrylate) (PMMA), poly(vinyl acetate) (PVA), poly(ethylene oxide) (PEO), poly(vinyl chloride) (PVC), poly(styrene-b-methyl methacrylate) di-block copolymer (PS-PMMA), as well as silver nano-particle and carbon nanotube-doped PMMA [17].

(a) Polymer solution on applicator   (b) Solution brushed across array   (c) Solvent dries forming suspended fibers
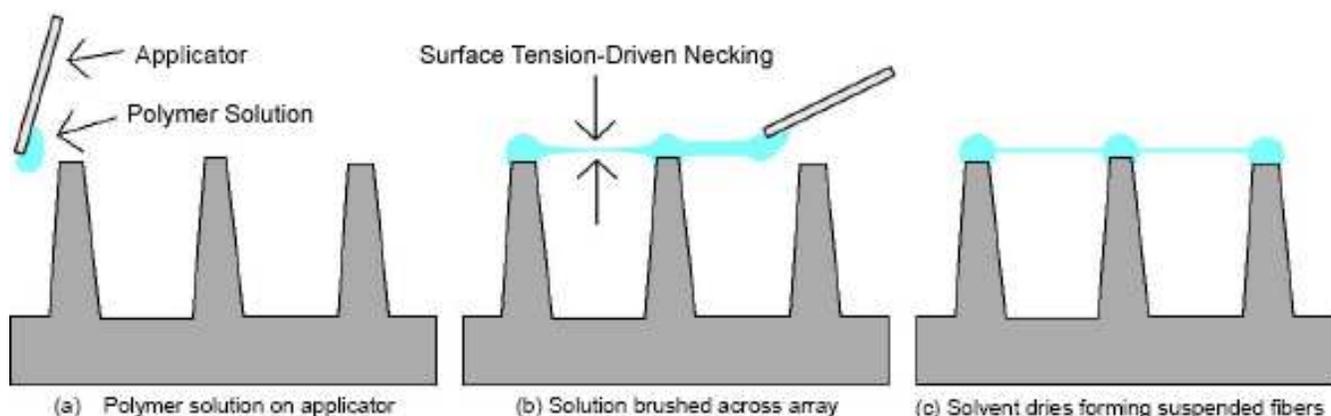
Figure 2 – Brush-on fiber fabrication process

The ability to fabricate fibers via brush-on from a particular polymer solution has been found to strongly correlate with the intrinsic properties of the solution [16]. Tripathi et al. have suggested that the likelihood of formation of a stable fiber from the surface tension-driven contraction of a column of polymer solution is related to a dimensionless "processability parameter", which is defined as

$$P = \frac{\eta h}{\sigma} \qquad [1]$$

where η is the shear viscosity of the polymer solution, h is the mass transfer coefficient of the solvent (which is directly correlated with solvent evaporation rate), and σ is the surface tension of the solution. It has been reported that P must exceed a minimum value in order to enable the fibers to solidify before the onset of capillary breakup. Specifically, stable macro- or microscale fibers will form upon instantaneous extension of solutions with values of P greater than 3 X 10^{-4} [18]. Additionally, we have fabricated unbroken micro- and sub-microscale fibers through non-instantaneous extension of polymer solutions with values of P as low as 4 X 10^{-6} [19]. Thicker fibers are formed by using solutions with higher values of P, which is accomplished by increasing either solution viscosity or solvent mass transfer coefficient of the solution (see Equation 1).

METHODOLOGY

PLLA-PEO-PLLA (136.4 kDa) was mixed with several solvents including toluene, chloroform, dimethyl formamide (DMF), and chlorobenzene. The solutions were visually screened in order to determine if complete dissolution of the polymer had occurred. Then a preliminary brush-on fiber fabrication was attempted using a variety of concentrations ranging from 10 to 20 wt. %. Previous studies had determined that concentrations of 13 to 20 wt. % are typically required to produce fibers [19].

Prior to the fiber processing experiments we evaluated the effects of annealing on morphology on thin films of the polymer. PLLA-PEO-PLLA 15 wt. % solutions in chloroform were spun on glass substrates at 1000 rpm for 10 seconds. SEM imaging revealed that films were 10-20 μm thick. These films were exposed to a variety of conditions including heating followed by gradual cooling, heating followed by quenching in water (18° C), and heating followed by gradual cooling and exposure to water. The films were coated with a thin (10-20 nm) layer of gold-palladium alloy for viewing them in the SEM (Zeiss Supra 35VP).

Parallel arrays of PLLA-PEO-PLLA fibers were constructed by manually brushing 20-40 μL volumes of polymer solution across arrays of pillars at velocities of 10 to 100 mm/s using a glass coverslip applicator. These pillars, which are square and measure 200 μm on a side, were fabricated through the selective machining of a glass slide with a dicing saw (Disco DAD321) (Figure 3). In addition to the pillar array region, the machined platforms possess multiple rectangular platforms on which cells can be easily seeded and cultured.
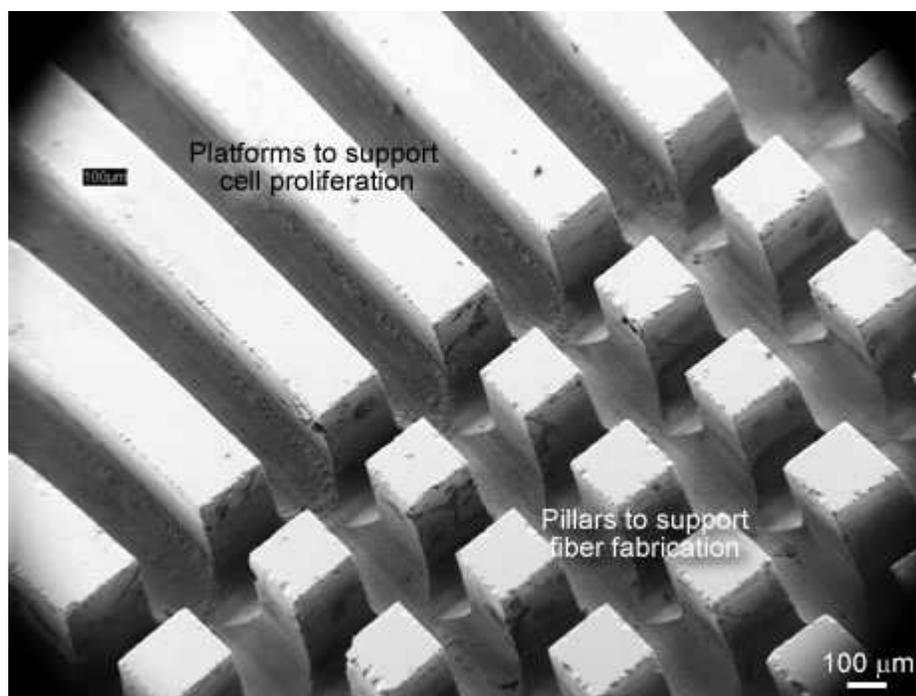

Figure 3 – Microfabricated arrays to support PLLA-PEO-PLLA fibers.

The effects of varying process conditions on the morphology of the suspended fibers were evaluated. The fiber arrays were heated to temperatures of 60° C, 100° C, and 140° C for 30 minutes and then transferred immediately into a water bath (18° C) for two minutes. Additionally, a longer immersion time of 15 minutes was

investigated to determine the effect of prolonged exposure to water on morphology. Morphology was evaluated by imaging gold-palladium coated samples in the SEM.

RESULTS AND DISCUSSION

Of the polymer-solvent solutions tested, 10 wt. % or greater solutions (necessary condition for stable formation of fibers) could only be produced with the solvents chloroform and DMF. Also, the lower volatility DMF evaporates much too slowly to prevent capillary breakup (Table I). As such, chloroform-polymer solutions were used for the studies of fiber formation and morphology.

Solvent	Solubility Limit	Brush-on Fiber Formation
Chloroform	Soluble	Yes
DMF	Soluble	No
Toluene	<10 wt. %	No
50:50 Toluene / Chloroform	<10 wt. %	No
Chlorobenzene	<10 wt. %	No

Table I – Preliminary evaluation of PLLA-PEO-PLLA solutions.

Films produced by spinning solvated PLLA-PEO-PLLA were exposed to the various heat and quenching treatments described in Table II. Prior to these treatments the polymer was smooth with a few isolated pores (ID 1). Polymer films treated with heat alone followed by a slow cooling in air (ID 2) or exposure to water alone (ID 3) were smooth with sparse cracking and isolated pores. However, when the polymer is heated to 140°C and then immediately quenched in room temperature water, an abundance of brittle fractures appear (ID 4). If instead the annealed sample is cooled slowly in air before exposure to water, the heavily fractured morphology is replaced by a sparsely fractured, but heavily porous structure (ID 5). Annealing the film at 180°C, followed by rapid cooling (ID 6) gives a morphology similar to ID 5.

ID	Treatment	Morphology
1	No treatment	Isolated Pores
2	140°C, 30 min Slow cool in air	Isolated Pores
3	Immerse in water, 2 min	Slight Cracking
4	140°C, 30 min Rapidly cool in water, 2 min	Significant Cracking and Isolated Pores
5	140°C, 30 min Slow cool in air for 20 minutes Immerse in water, 2 min	Very Porous and Slight Cracking
6	180°C, 30 min Rapidly cool in water, 2 min	Very Porous and Significant Cracking

Table II – Summary of treatments and resulting morphologies of PLLA-PEO-PLLA thin films

The film processing treatments in Table II result in two distinct morphologies 1) cracks (Figure 4a) and 2) nanoscale pores (Figure 4b). The cracks are most abundant following rapid cooling which is causing considerable contraction and large stresses which are likely causing brittle fracture. The pores are most abundant when the film is heated and then immersed in water (ID 5 and ID 6). The density of pores does not appear to be much affected by whether the cooling is rapid or slow. A possible mechanism for the pore formation is phase separation between the PEO and PLLA domains occurring when the polymer is heated above the glass transition temperature of the constituents (T_g PEO = -115 to -40° C, T_g PLLA = 53 to 64° C [20]), enabling increased polymer chain motion and selective growth of phase-separated domains. On contact with water, the hydrophilic and water-soluble PEO domains swell as water diffuses into them while hydrophilic PLLA domains shrink away from the water. As the polymer dries, the PEO regions contract resulting in a porous morphology.
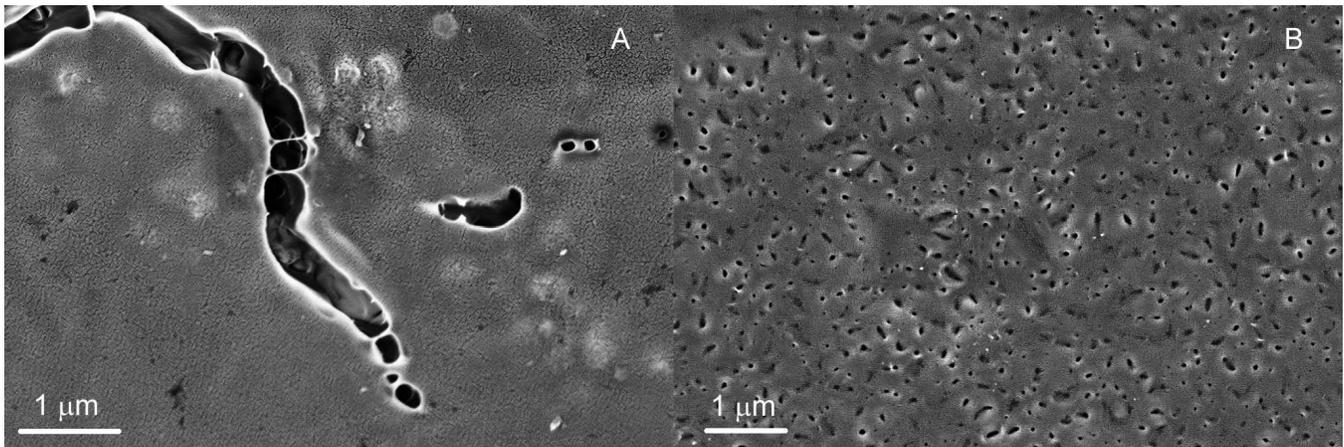
Figure 4 – a) Brittle fracture induced by rapid cooling of an annealed film (ID 4); b) Nanopores induced by water exposure after air cooling an annealed film (ID 5).

Arrays of suspended PLLA-PEO-PLLA fibers were drawn between the microfabricated glass pillars by the brush-on method (Figure 5). The fibers were measured to have diameters ranging from 125 nm to over 100 µm with the vast majority of fibers falling between 3 to 15 µm, encompassing the typical diameter of vascular capillaries, which generally range from 5 to 10 µm in diameter [21]. Untreated fibers and fibers that were heated, but not exposed to water, were observed to have smooth surface morphologies (Figures 6a and 6b). Fibers treated only with water showed a small degree of cracking that was generally aligned along the axis of the (Figure 6c). Fibers heated to only 60° C prior and cooled in water were m ostly smooth with a small amount of cracking and pitting. Fibers rapidly cooled in water after annealing at 140° C usually broke apart due to excessive fractures. The remnants of the fibers showing numerous small cracks or voids (Figure 6d). Fibers annealed to 100° C and rapidly cooled in water had shallow surface features that were generally aligned around the circumference of the fiber (Figure 6e). After increasing the water exposure time from 2 minutes to 15 minutes, the shallow indentions had been replaced by pronounced, circumferentially-oriented pores (Figures 6f). Measurement of a representative sample of these pores indicated that pore size measured 393.8 ± 448.5 nm in the circumferential direction and 103.9 ± 55.5 nm in the axial direction.
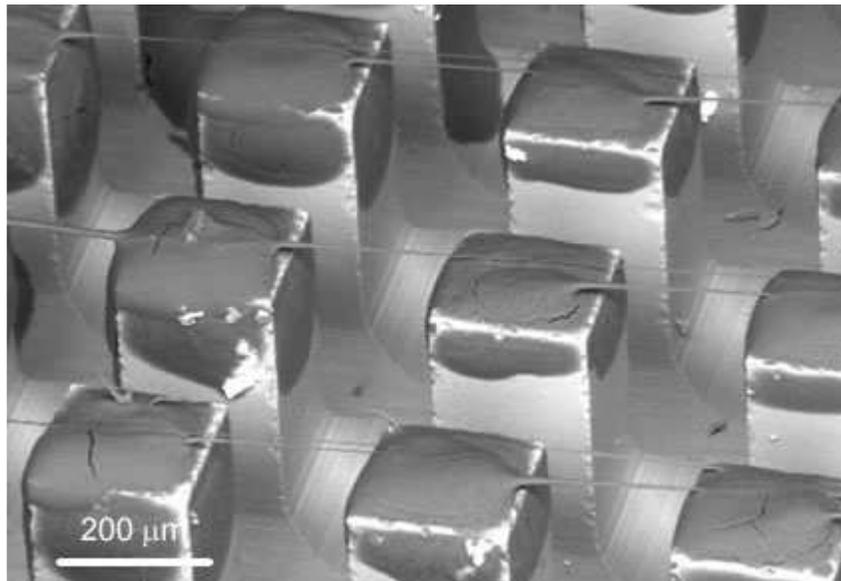

Figure 5 – Suspended PLLA-PEO-PLLA fibers fabricated by the brush-on technique. A 15 wt. % polymer-solvent solution was used. The fibers shown are around 10 microns diameter.
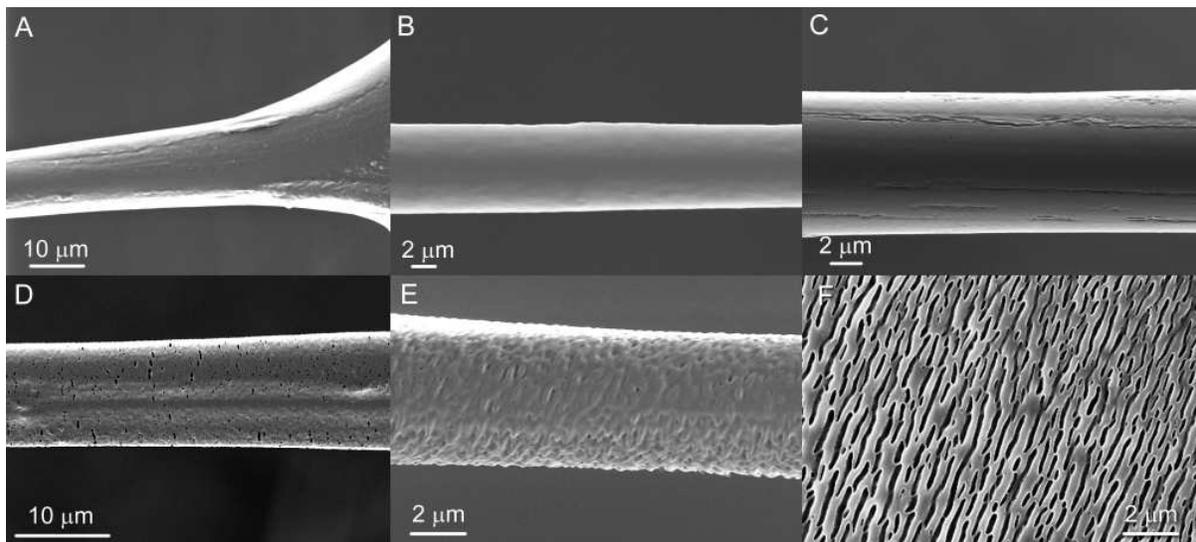
Figure 6 – Various surface morphologies generated by various treatments: a) Prior to treatment, b) heat treatment alone at 140 °C, c) water exposure alone, d) anneal ed at 140 °C followed by rapid cool, e) annealing at 100°C moderate temperature followed by rapid cool, f) annealing at 100 °C followed by slow cool.

SUMMARY

The brush-on technique was used with to fabricate arrays of suspended fibers of PLLA-PEO-PLLA having diameters from 125 nm to 50 microns. Thermal and water treatments applied to these fibers changes the morphology from smooth to nanoporous. Under specific treatment conditions the pores can be circumferentially-aligned. Based on the ability of the polymer to form such surface morphologies, combined with the polymer's innate biodegradability, biomimetic mechanical properties, and capacity to absorb and release biomolecules, the fibers appear to be very appropriate and interesting candidates for synthetic ECMs. The ability of the brush-on technique to form the polymer into suspended arrays of fibers of a few microns diameter makes them especially well-suited for scaffolds for tissue generation of microvascular networks, which are critically needed for the regeneration of complex tissues and organs.

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