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Electrospinning of Biosyn®-based tubular conduits: Structural, morphological, and mechanical characterizations

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ABSTRACT

Electrospinning has garnered special attention recently due to its flexibility in producing extracellular matrix-like non-woven fibers on the nano-/microscale and its ability to easily fabricate seamless three-dimensional tubular conduits. Biosyn®, a bioabsorbable co-polymer of glycolide, dioxanone, and trimethylene carbonate, was successfully electrospun into tubular conduits for the first time for soft tissue applications. At an electric field strength of 1 kV cm^{-1} over a distance of 22 cm (between the Taylor cone and the collector) and at a flow rate of 1.5 ml h^{-1} different concentrations of Biosyn/HFP solutions (5–20%) were spun into nanofibers and collected on a rotating mandrel (diameter 4 mm) at 300 and 3125 r.p.m. Scaffolds were characterized for structural and morphological properties by differential scanning calorimetry and scanning electron microscopy and for mechanical properties by uniaxial tensile testing (in both the circumferential and longitudinal directions). Biosyn® tubular scaffolds (internal diameter 4 mm) have been shown to exhibit a highly porous structure (60–70%) with a randomly oriented nanofibrous morphology. The polymer solution concentration directly affects spinnability and fiber diameter. At very low concentrations (⩽5%) droplets were formed due to electrospraying. However, as the concentration increased the solution viscosity increased and a "bead-on-string" morphology was observed at 10%. A further increase in concentration to 13% resulted in "bead-free" nanofibers with diameters in the range 500–700 nm. Higher concentrations (⩾20%) resulted in the formation of microfibers (1–1.4 μm diameter) due to increased solution viscosity. It has also been noted that increasing the mandrel speed from 300 to 3125 r.p.m. produced a reduction in the fiber size. Uniaxial tensile testing of the scaffolds revealed the mechanical properties to be attractive for soft tissue applications. As the fiber diameters of the scaffold decrease the tensile strength and modulus increase. There is no drastic change in tensile properties of the scaffolds tested under hydrated and dry conditions. However, a detailed study on the biodegradation and biomechanics of electrospun Biosyn conduits under physiological pressure conditions is required to ensure potential application as a vascular graft.

1. Introduction

Scaffold-based tissue engineering can be considered to be the "holy grail" for the regeneration of damaged tissues and organs. A synthetic vascular scaffold must resemble the characteristics of the native extracellular matrix (ECM), including nanoscale size, mechanical integrity and favorable bioabsorbability and biocompatibility [1]. Collagens, a major constituent of the ECM, have fibers in the diameter range 50–500 nm [2]. Electrospun non-woven fibers with diameters in the nanoscale range morphologically resemble the nanofibrillar ECM proteins. Cells are responsive to the nanotopography of polymer nanofibers that cells attach to and organize around fibers with diameters smaller than that of cells [3,4]. Tissue engineered vascular graft research has been rejuvenated in recent years, due in part to the ability to create ECM-mimicking scaffolds through techniques such as self-assembly, phase separation and electrospinning.

Electrospinning has gained much popularity recently as an enabling nanotechnology process for making seamless tubular scaffolds of various diameters and lengths from an assortment of synthetic polymers and biopolymers for vascular graft applications [5–8]. Apart from its simplicity, electrospinning offers the potential to tailor the composition, structure, and mechanical properties of scaffolds by controlling the solution composition/properties and spinning conditions [8,9]. Moreover, tubular conduits of various

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diameters and lengths needed for vascular reconstructions (i.e. ~15 cm length for coronary bypass and 15 to >30 cm for peripheral reconstructions) can be easily fabricated by depositing fibers onto rotating mandrels of desired diameters and lengths [10]. The grafts can be tailored to be flexible and strong enough to withstand pressurized physiological conditions, thereby exhibiting properties that resemble native blood vessels [11,12]. Reports have shown that electrospun biodegradable scaffolds seeded with mesenchymal stem cells have long-term patency when implanted into rat carotid arteries using a bypass procedure [13]. Our own recent studies have indicated the formation of a functional human endothelium on the lumen of an electrospun scaffold of protein/polymer blends [14]. It is believed that the nanoscale dimension of electrospun scaffolds provides a well-defined architecture with a high surface to volume ratio which promotes cell adhesion and growth and accelerates subsequent tissue regeneration more than any other known scaffold form [15]. Electrospun scaffolds also display a high porosity, with highly connective pores for the exchange of cell nutrients and metabolic waste. Fabrication of the outer layer of the tubular scaffold with circumferentially aligned fibers can orient smooth muscle cells (SMC) in a manner similar to that observed in blood vessels. Recent developments, such as simultaneous electrospraying of SMC during the spinning of elastomeric poly(ether urethane urea) (PEUU), rather than seeding cells on the scaffold, yielded a small diameter conduit with uniform integration of cells [11]. This simultaneous "cell microintegration" while electrospinning nanofibers overcomes the inherent challenge in obtaining cell infiltration through the small pores of electrospun scaffolds observed during cell seeding.

Many bioresorbable polymers, alone or in conjunction with vascular proteins, have been experimentally explored for the construction of small diameter tubular scaffolds by electrospinning. Drilling et al. [16] have used polycaprolactone (PCL) for the fabrication of a burst pressure-competent vascular graft via electrospinning. Pektok et al. [17] have reported that electrospun PCL-based small diameter grafts (internal diameter 2 mm) showed better healing characteristics and faster endothelialization in vivo than an expanded polytetrafluoroethylene counterpart. Luong-Van et al. [18] incorporated heparin into electrospun PCL to prevent the migration of SMC into the lumen layer. Recently, a composite graft of collagen and PCL has been reported by Lee et al. [12]. Kwon et al. [19] electrospun a small diameter graft (internal diameter 3 mm) from poly(lactide-co-caprolactone) (PLCL). A blend of this polymer with collagen showed enhanced growth of endothelial cells (EC) compared with neat PLCL [20]. Jeong et al. [21] have co-electrospun marine collagen and poly(lactide-co-glycolide) (PLGA) into tubular scaffolds (internal diameter 3 mm) and seeded with EC and SMC in a perfusion bioreactor. Stitzel et al. [22] have created tubular scaffolds based on ternary blends of collagen, elastin and PLGA having compliances similar to native artery. A unique approach of functionally graded tubular scaffolds of spatially designed layers by sequential co-spinning of ternary blends of gelatin, elastin and polyglyconate or polydioxanone was reported by Thomas et al. [10,23] to mimic the heterogeneously layered structure of native blood vessels. Polydioxanone (PDO), an absorbable suture material having more flexibility than rigid polylactic acid (PLA) or polyglycolic acid (PGA) due to the presence of an additional ether group in its structure, has recently been explored in vascular matrix applications. Sell et al. [24] have electrospun elastin-containing PDO grafts to mimic the biomechanics of native arteries. Blends of PDO with PCL and silk fibroin were also electrospun to evaluate its potential for vascular applications [25].

Although many biodegradable polymers have been spun into fibers to make potential nanofibrous scaffolds for vascular grafts as stated earlier, the continuous search for a "safe and biocompatible" scaffolding material has tempted us to electrospin clinically proven suture materials. Biosyn®, a tri-co-polymer of glycolide, dioxanone and trimethylene carbonate, could be a promising material of choice for vascular applications as it possess soft tissue mechanical properties, optimum biodegradability [26], favorable biocompatibility, and to some extent, shape memory characteristics. Tubular conduits (internal diameter 4 mm) were fabricated under optimized spinning conditions and characterized for structural, morphological and mechanical properties. This work also reports for the first time the electrospinning of Biosyn into nanofibers.

2. Materials and methods

2.1. Materials

Biosyn® was obtained in the form of monofilament surgical sutures (Advanced Inventory Management Inc., Mokena, IL) and 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) was purchased from Sigma–Aldrich (St. Louis, MO). Chemically, Biosyn is poly(glycolide-co-dioxanone-co-trimethlene carbonate) with a composition of 60% glycolide, 14% dioxanone and 26% trimethylene carbonate, as shown in Scheme 1. The center block is a random co-polymer of 1,3-dioxane-2-one (65 wt.%) and 1,4-dioxane-2-one (35 wt.%). Biosyn has a reduced stiffness compared with highly crystalline pure PGA, with absorption within 3–4 months [27].

2.2. Solution preparation and viscosity measurements

Biosyn® was dissolved in HFP to form solutions with concentrations varying from 5% to 20% and thoroughly homogenized by magnetic stirring. Changing the polymer concentration can vary the solution viscosity. Solution viscosities were measured using a constant stress rheometer with parallel plate geometry (TA Instruments AR 1000-N). Each experiment was performed at room temperature using 4 cm diameter parallel plates having a thin layer of polymer solution between them.

2.3. Electrospinning and process optimization

Solution concentration, feeding rate, voltage and distance were optimized to create scaffolds. Biosyn/HFP concentrations at 5%, 8%, 10%, 13%, 15% and 20% w/v were electrospun onto a static collector (0 r.p.m.) and viewed under an optical microscope to optimize the concentrations giving the best likelihood of producing bead-free fibers. A high voltage of 22 kV was applied using a high voltage power supply (M826, Gamma High-Voltage Research, Ormond Beach, FL). Solutions were ramped at a rate of 1.5 ml h^{-1} using a syringe pump (PHD 2000, Harvard Apparatus) fitted with a BD 3 ml syringe having a 27 gage needle (Small Parts Inc.). The electrically charged polymer jet ejected from the needle is directed towards the grounded collector placed 22 cm away from the needle and deposited there as fine fibers. To optimize the critical spinning conditions, a feeding rate between 0.5 and 2 ml h^{-1} and a range of electrical potential of 15–22 kV were used, while keeping the other variables constant.

2.4. Fabrication of tubular conduits

A stainless steel rod of 4 mm diameter was set in a rotating drill, leaving approximately 20 cm exposed for the collection of fibers in the case of fabrication of tubular conduits. Biosyn concentrations of 10, 13, 15 and 20 wt.% were spun under optimized spinning conditions of 1.5 ml h^{-1} feed rate and 22 kV voltage onto a rotating collector 22 cm away from the spinneret needle (27 gage) at 300 and 3125 r.p.m. under ambient conditions. The scaffolds were recovered from the mandrel, desiccated in vacuum for 48 h, and optical

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