



Fabrication of a model continuously graded co-electrospun mesh for regeneration of the ligament–bone interface

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ABSTRACT

Current scaffolds for the regeneration of anterior cruciate ligament injuries are unable to capture intricate mechanical and chemical gradients present in the natural ligament–bone interface. As a result, stress concentrations can develop at the scaffold–bone interface, leading to poor osseointegration. Hence, scaffolds that possess appropriate mechano-chemical gradients would help establish normal loading properties at the interface, while promoting scaffold integration with bone. With the long-term goal of investigating regeneration of the ligament–bone interface, this feasibility study aimed to fabricate a continuously graded mesh. Specifically, graded meshes were fabricated by co-electrospinning nanohydroxyapatite/polycaprolactone (nHAP-PCL) and poly(ester urethane) urea elastomer solutions from offset spinnerets. Next, mineral crystallites were selectively deposited on the nHAP-PCL fibers by treatment with a 5× simulated body fluid (5× SBF). X-ray diffraction and energy-dispersive spectroscopy indicated calcium-deficient hydroxyapatite-like mineral crystallites with an average Ca/P ratio of 1.48. Tensile testing demonstrated the presence of a mechanical gradient, which became more pronounced upon treatment with 5× SBF. Finally, biocompatibility of the graded meshes was verified using an MC3T3-E1 osteoprogenitor cell line. The study demonstrates that graded meshes, for potential application in interfacial tissue engineering, can be fabricated by co-electrospinning.

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1. Introduction

Despite significant advances in tissue engineering for the repair of anterior cruciate ligament (ACL) tears and ruptures [1,2], a concern with current regeneration strategies is poor integration of the engineered ligament with bone [3,4]. Weak osseointegration of scaffolds stems from the presence of sharp gradients in mechanical, chemical and biological properties at the natural ligament–bone interface [5,6]. Homogeneous scaffolds do not capture the intricate gradients from soft unmineralized tissue to hard mineralized tissue found at bony insertion sites and rely on post-operative healing to generate these gradients. Therefore, a scaffold that possesses gradients in mechanical properties and chemical composition is anticipated to integrate better with bone and help regenerate the interfacial tissue.

Graded scaffolds that mimic the ligament to bone transition have been the subject of recent efforts. Cooper et al. [7] designed

a three-dimensional (3-D) fiber-based woven scaffold consisting of a femoral attachment region, a ligament region and a tibial attachment region. The scaffold possessed gradients in porosity, pore size and fiber orientation along its length to promote good integration with the femoral and tibial tunnels. Spalazzi et al. [8,9] fabricated triphasic scaffolds using poly(lactide-co-glycolide) (PLGA) and bioactive glass for the regeneration of the ligament to bone transition. The biodegradable polymer component was employed to help support cell attachment and proliferation, while the bioactive glass was used for its osteoinductive property. While these and previous efforts have produced graded scaffolds by combining multiple phases into a composite, the fabrication of continuously graded scaffolds with appropriate geometry and chemistry has not been achieved. The choice of scaffolding technique in particular will play an important role in determining scaffold geometry and morphology, which in turn may have a significant impact on tissue regeneration.

Among the various scaffolding techniques available, electrospinning has been widely used chiefly because of its ability to create fairly complex non-woven meshes [10,11]. Specifically, it has great potential for developing engineered ligament tissue, as aligned fibers in the meshes can provide appropriate cues to cells through

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contact guidance [12]. Electrospinning from two or more spinnerets, known as co-electrospinning, has been studied in recent years due to the increased level of flexibility it offers in creating meshes with complex geometries and hybrid properties. For example, Baker et al. [13] created a porosity gradient by co-electrospinning poly(ϵ -caprolactone) (PCL) and poly(ethylene oxide) (PEO) from offset spinnerets and subsequently leaching out the water-soluble PEO nanofibers. In another study, Li et al. [14] created blends of natural and synthetic polymeric scaffolds by co-electrospinning solutions of gelatin, elastin and PLGA in two different ratios from separate spinnerets. Co-electrospinning can also be used to fabricate continuously graded scaffolds for the regeneration of orthopedic interfaces. In a recent study, Ladd et al. [15] co-electrospun polymers from offset spinnerets to create a mechanical gradient for engineering the muscle–tendon junction. This approach has the advantage of controlling scaffold chemistry by using polymers with different chemical properties to create spatial gradients in chemistry. Further, the selective incorporation of mineral into one set of fibers to create a mineral gradient may aid the regeneration of the ligament–bone interface.

A few strategies have been employed for the fabrication of mineral gradients towards regeneration of orthopedic interfaces. Such methods have involved the use of a twin-screw extrusion device to deliver different amounts of polymer and hydroxyapatite [16,17] and the inclusion of $\text{CaCO}_3/\text{TiO}_2$ nanoparticles into 3-D PLGA microsphere scaffolds [18]. Another approach for incorporating a mineral gradient is the immersion of scaffolds in a simulated body fluid (SBF) [19]. SBF is a supersaturated solution that deposits hydroxyapatite-like mineral on various surfaces [20]. Studies in the literature have reported that the use of higher concentrations, such as $5\times$ SBF and $10\times$ SBF, increases the rates of mineralization [21–24]. Higher concentrations also result in the incorporation of impurities in the mineral due to cationic substitutions for calcium. However, the presence of minor amounts of impurity may improve solubility and bioactivity of the mineral in the context of bone tissue formation. Previously, a mineral gradient has been formed by exposing various sections of a scaffold to a $5\times$ SBF solution for different time periods [19]. However, an approach that exploits the surface chemistry of different polymers to nucleate and grow different amounts of mineral from SBF solutions is intrinsically simpler and may result in better reproducibility.

The aim of this study was to fabricate meshes possessing model mechanical and mineral gradients that could be used to study regeneration of the ligament–bone interface. PCL and a custom-made poly(ester urethane) urea elastomer (PEUUR2000) were employed to create meshes that could potentially help in the integration of the scaffold with bone and ligament tissues, respectively. PCL was doped with nanohydroxyapatite particles (nHAP) and co-electrospun with PEUUR2000 from offset spinnerets to form graded meshes. To create mineral gradients, meshes were treated with a $5\times$ SBF solution that selectively deposited crystallites of hydroxyapatite-like mineral on nHAP-PCL fibers. The mineral composition, water contact angles and mechanical properties of different regions of the meshes were measured to characterize the gradient properties, and mammalian cell culture was performed to confirm cell metabolic activity.

2. Materials and methods

2.1. Materials

All chemicals and laboratory supplies were purchased from Fisher Scientific (Pittsburgh, PA) and biological supplies from Invitrogen (Gaithersburg, MD), unless otherwise noted. PCL (inherent viscosity: 1.15 dl g^{-1}) was purchased from LACTEL biodegradable

polymers (Birmingham, AL) and 2,2,2-trifluoroethanol was purchased from Acros Organics (Morris Plains, NJ). 1,1,1,3,3,3-Hexafluoro-2 propanol and nHAP particles ($<200 \text{ nm}$) were purchased from Sigma–Aldrich (St. Louis, MO).

2.2. Polyurethane synthesis

A linear segmented degradable poly(ester urethane) urea elastomer (PEUUR2000) was synthesized using a standard two-step technique. Briefly, a 2000 Da PCL diol was end-capped with 1,6-diisocyanatohexane and then chain extended with 1,3-propanediol bis(4-aminobenzoate). The detailed procedure for this synthesis is described elsewhere [12].

2.3. Electrospinning of graded meshes

PCL was dissolved in 2,2,2-trifluoroethanol at 12% (w/v) and nHAP particles were added to the solution at 3% (w/v). Fluorescent dye 1,1'-dilinoleyl-3,3',3'-tetramethylindocarbocyanine perchlorate (DiI; Invitrogen) was added to the solution at a concentration of $5 \mu\text{g ml}^{-1}$ and the solution/suspension was stirred vigorously for 2 days to dissolve the PCL and disperse the nHAP particles. PEUUR2000 was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol at 14 wt.% and doped with fluorescent dye 3,3'-dilinoleylloxycarbocyanine perchlorate (DiO; Invitrogen) at a concentration of $8 \mu\text{g ml}^{-1}$ and the solution was stirred for two days.

Prior to electrospinning, a mandrel was wrapped in aluminum foil and three 25 mm circular glass cover slips (sonicated in acetone and air-dried) were attached with double-sided tape to the foil along its length. Solutions of nHAP-PCL and PEUUR2000 were loaded into two 10 ml plastic syringes, capped with 22 gauge Teflon-tipped needles and placed at opposite ends of the mandrel. The grounded mandrel was rotated at 1.2 ms^{-1} and nHAP-PCL was electrospun using a +20 kV potential, a throw distance of 20 cm and a flow rate of 2 ml h^{-1} , while PEUUR2000 was electrospun using a +15 kV potential, a throw distance of 15 cm and a flow rate of 3 ml h^{-1} . Subsequently, the syringes were offset 7 cm along the length of the mandrel (Fig. 1) to achieve a gradient in fiber deposition. After a few minutes of electrospinning, the coverslips were detached from the mandrel and imaged under an Olympus IX50 inverted microscope (Opelco, Sterling, VA), equipped with a digital camera (Model C4742-95, Hamamatsu, Bridgewater, NJ). Electrospinning was continued for over an hour to obtain a graded mesh. In order to achieve uniform thickness along the length of the mesh, electrospinning was performed roughly twice as long on the pure nHAP-PCL and PEUUR ends as compared to the transition region in the middle. For ease of processing, the graded mesh was divided into three regions: a region consisting only of nHAP doped PCL fibers (nHAP-PCL(u)); another consisting solely of PEUUR2000 fibers (PEUUR2000(u)); and a transition consisting roughly of a 50:50 mix of both types of fibers (GRAD(u)). The samples were stored in a desiccator until further use.

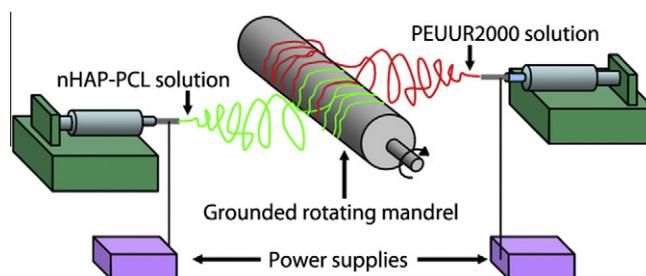


Fig. 1. Diagram of electrospinning apparatus depicting offset spinnerets.

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