Integrated Dual Scaffolding System for Engineering of Muscle-Tendon Junctions

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Statement of Purpose: While tissue engineering has had initial successes with building simple homogeneous tissues, there is an increasing demand for developing composite tissue systems that require coordinated function. This is especially evident in musculoskeletal tissue systems where muscle and tendon must be conjoined in order to effectively transmit forces from muscle to tendon. One challenge in developing such tissues is designing a single scaffold that can accommodate the unique mechanical properties of the different tissue types. In this study we developed a continuous, integrated, dual scaffolding system using a co-electrospinning fabrication technique that had regional variations in mechanical properties. To our knowledge, this is the first report of the development of such a scaffolding system.

Methods: Two different polymer solutions, 10% (w/v) poly(ε-caprolactone)/collagen and 5% (w/v) poly(L-lactide)/collagen blends with the ratio of 1:1 in weight, were simultaneously electrospun (using high voltage power at 20 kV potential) onto opposite ends of a cylindrical mandrel to create a scaffold with 3 distinct regions: a PCL/collagen side (PCL side), PLLA/collagen side (PLLA side), and a center overlap region (Fig. 1). Both solutions were delivered through a blunt tip at a constant flow rate of 1 mL/hr using a syringe pump. The distance between the syringe tip and the mandrel was 10 cm and the rate of rotation was 1000 rpm.

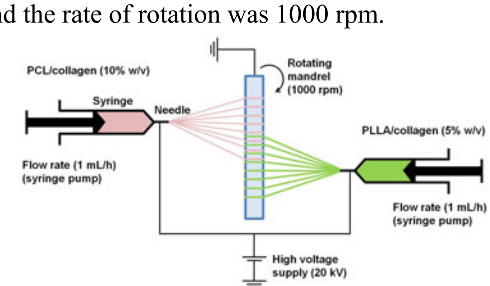

Figure 1. Schematic of the dual-electrospinning set-up used to fabricate the integrated dual scaffolding system.

Subsequently, the scaffolds were cross-linked with 2.5% glutaraldehyde vapor for 2 hr. Characterization of the scaffolds included ultrastructural morphology (n=3), uniaxial tensile testing (n=6), cyclic tensile testing (n=6), and stress relaxation testing (n=6). The quasi-linear viscoelastic (QLV) model was used to describe the scaffold's viscoelastic behavior. The scaffold was tested for biocompatibility and seeded with C2C12 myoblasts to demonstrate its accommodation of myotube formation.

Results: The results demonstrate that an integrated, dual scaffolding system can be created using co-electrospinning that is biocompatible, displays a nanofiber architecture with fiber diameters ranging from 505-606 nm, exhibits vast regional variations in mechanical properties with moduli ranging from 3.41-24.35 MPa, and withstands cyclic mechanical and stress-relaxation testing.

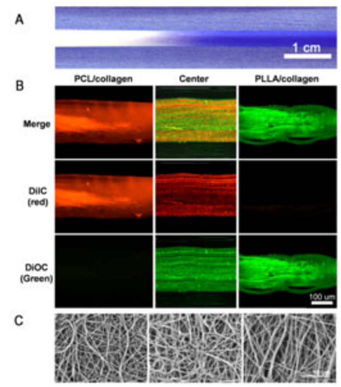

Figure 2. (A) The dual scaffolding system displayed overlap. Methylene blue was added to PLLA side in order to show contrast between the two materials. (B) Fluorescent dyes were incorporated into PCL/collagen (red) and PLLA/collagen (green) to show the overlap at a fiber level. (C) SEM images (4,000×) of the different regions; PCL side, center, and PLLA side.

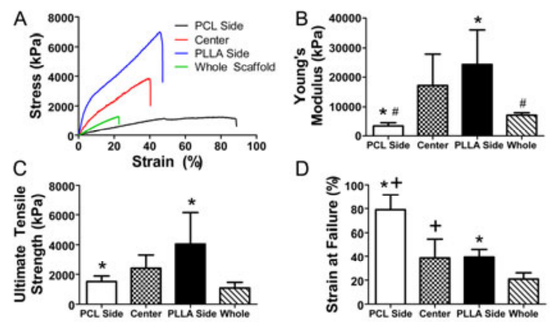

Figure 3. (A) Representative stress-strain curves of the different regions and whole scaffold under tensile testing to failure. (B) Young's modulus, (C) ultimate tensile strength, and (D) strain at failure of the different regions and whole scaffold showed the distinct differences in mechanical properties between regions. $*,\dagger,\#$ $P < 0.05$ (n=6).

The scaffolds were successfully characterized using the QLV model. The following parameters were obtained using the model: A=18,050,000 kPa, B=3.408×10^{-6}, C=0.09561, τ_1=0.00814, and τ_2=3657.53. These parameters are similar to values obtained for scaffolds consisting of similar materials and will allow comparison to other scaffolds and tissues.

Conclusions: We have described the development of a novel, integrated, dual scaffolding system that has distinct mechanical properties within different regions of the system. Characterization with cyclic and stress-relaxation testing showed that the dual-scaffolding system has good mechanical properties and viscoelastic properties that mimic the pattern of properties exhibited by that of a native muscle-tendon unit. The scaffold is biocompatible, accommodates C2C12 myoblasts, and allows myotube formation. This system may serve as an excellent scaffold for the formation of MTJ composite tissues. Thus, the properties engineered into these scaffolds make them attractive candidates for use in the formation muscle-tendon junctions. Future studies will investigate the ability of these scaffolds to form MTJ tissue and restore function *in vivo*.