Co-electrospun Scaffolds with Gradients in Fiber Alignment and Chemistry for the Regeneration of Ligament-Bone Transitions

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Statement of Purpose: Grafts currently used for the repair of ligament injuries integrate poorly with bone due to a significant mismatch of mechanical and chemical properties between graft and bone. This mismatch stems from the grafts' inability to regenerate gradients of mineral content, collagen fiber alignment and cell phenotypes that exist at natural ligament-bone transitions. We envision that an electrospun scaffold, possessing continuous gradients of architecture, mechanical and biochemical properties, will help establish spatial gradients of cell phenotype in bone marrow stromal cells (BMSCs) and thus aid the regeneration and osseointegration of injured ligaments. As a step towards the larger goal of regenerating complex tissue transitions, we describe herein the fabrication of a scaffold possessing gradients of mineral content and fiber alignment, obtained by co-electrospinning two polymer solutions from offset spinnerets onto a dual-drum collector. The dual-drum collector used in this study allows the creation of graded scaffolds with three distinct regions at physiologically relevant length-scales.

Methods: Two solutions of polycaprolactone (PCL), one doped with tricalcium phosphate (TCP) particles, were co-electrospun simultaneously from offset spinnerets onto a custom-designed slowly rotating dual-drum collector possessing a 2.5 cm gap region between the drums (**Fig.1**) such that the PCL fibers aligned in the gap region, TCP-doped PCL fibers deposited randomly onto one of the drums and a mixture of fibers from both spinnerets formed a transition zone in between. This process resulted in the formation of a scaffold possessing gradients in fiber alignment and chemistry.

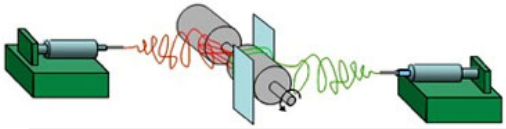

Fig.1: Apparatus depicting offset spinnerets and polymers being co-electrospun onto a dual-drum collector.

Angular orientation and diameter of fibers from the three regions was characterized from micrographs of the graded scaffold. Rat BMSCs were seeded on all regions of the graded scaffold and cell alignment, metabolic activity, deposition of collagen-I (col-I) and phenotypic makers of cell differentiation were investigated.

Results: Micrographs from the three different regions of the graded scaffold revealed a gradient of fiber alignment and TCP content (**Fig.2**). Fiber alignment was quantified by defining an angular standard deviation for each region.

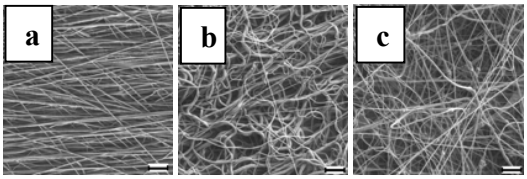

Fig.2: Micrographs of (a) aligned PCL fibers, (b) fibers from the interfacial region and (c) randomly oriented TCP-loaded PCL fibers. Scale bars represent 10 microns.

After 3 days of culture, rat BMSCs were found to be aligned in the aligned region of the scaffold and randomly oriented on the other regions (**Fig.3**). Moreover, actin cytoskeleton was found to be bundled in the cells on the aligned region compared to the diffuse actin in the cells on the random region. Cells were also found to be metabolically active on all regions of the scaffold after 1 and 7 days of culture. Finally, quantification of mRNA expression after 7 and 14 days of culture revealed a gradient of cell phenotype and immunohistochemical staining showed a gradient of col-I orientation.

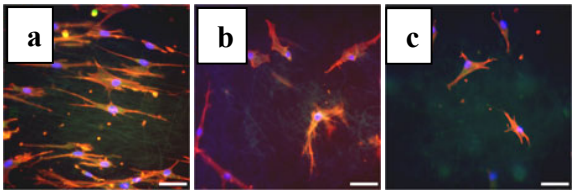

Fig.3: BMSCs on (a) aligned PCL region, (b) interfacial region and (c) randomly oriented TCP-loaded PCL region stained for actin (red), vinculin (green) and nuclei (blue). Scale bars represent 50 microns.

Conclusions: This study demonstrates that scaffolds with complex architectures and gradients in chemical properties at physiologically relevant length-scales, can be fabricated by co-electrospinning appropriate polymer solutions from offset spinnerets onto a specially designed collector. The study also indicates that these scaffolds have the potential to be used for the regeneration of graded tissue interfaces such as those present at ligament-bone transitions. Efforts are currently underway to create a gradient of fiber chemistry and mechanical properties by co-electrospinning polymers with significantly different mechanical properties, as well as spatially varying the fiber diameter in the different regions of the scaffolds to further influence cell differentiation.