Spatially-Patterned Collagen Scaffolds for Orthopedic Tissue Regeneration

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Introduction

Tendons are specialized connective tissues that transmit tensile loads between bone and muscle; of particular need are materials for tendon-bone-insertion (TBI) injuries [1]. We have recently developed collagen-glycosaminoglycan (CG) scaffold variants for osteochondral and bone applications [2]. Our goal is to develop multi-scale, hierarchical CG scaffolds with spatially tunable chemical, microstructural, and mechanical properties to mimic the tendinous, osseous, and interfacial TBI compartments.

Materials and Methods

Composites were created from CG scaffolds and dense CG membranes from a suspension of type I collagen and chondroitin 6-sulfate [3]. CG membranes were created via an evaporative process; CG scaffolds were fabricated by lyophilization [3]. Freezing temperature and mould thermal conductivity mismatches were used to create aligned pores [3]. Scaffold microstructure was assessed via stereology [3]. Tensile testing was performed using an MTS Insight 2. A benzophenone (BP) method recently developed to photochemically pattern 2D substrates [4] was modified to enable BP functionalization of the CG scaffold. An Ar+ Ion laser with photomask was used to expose regions of the scaffold to enable BP-mediated attachment of concanavalin A-biotin; patterns were visualized via streptavidin-QD525 using an LSM 710 confocal microscope. MC3T3-E1 cell viability was assayed at 48hr [3]. ANOVA and pair-wise multiple comparison procedures were used (significance: p < 0.05).

Results

CG scaffolds showed longitudinally aligned, pores (Fig. 1). Mean pore sizes of 42±4μm and 93±13μm (Mean±SD) were observed for liquid nitrogen or -60°C solidification, respectively. Pore anisotropy (aspect ratio: AR) in the longitudinal plane (AR: 1.6±0.1) was significantly greater than transverse (1.1±0.04, p<0.01). Tensile (hydrated) moduli were determined for: CG scaffolds (0.5% rel. density) – 25±10kPa; CG membranes (23±3 m thick; 79% rel. density) – 47±10MPa. CG scaffold-composites had ~15-fold increase in tensile modulus over scaffold. Optimization of membrane thickness is being performed to further increase modulus. BP photopatterning enabled creation of spatially-patterned surface chemistry profiles in the scaffold (full-thickness, 100 m wide stripes, 500 m periodicity; Fig. 2). 48 hr cell viability in BP-treated versus untreated CG scaffolds was comparable. Tenocyte and osteoblast viability in Fibronectin-functionalized CG scaffolds is ongoing.

Discussion and Conclusions

Here we describe development of longitudinally-aligned CG scaffolds, addition of a CG membrane to increase tensile strength, and spatial patterning of chemical moieties within a CG scaffold. Future work will optimize pore microstructure, mechanical properties, and chemical patterning to improve speed of cellular in-growth as well as resultant cell viability, alignment, and ECM protein biosynthesis.

References


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