Aligned and Conductive 3D Collagen Scaffolds for Skeletal Muscle Tissue Engineering

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Statement of Purpose: The cellular microenvironment modulates many cell behaviors including cell-cell signaling, cell spreading, proliferation, and differentiation. Bioelectrical stimuli in the microenvironment can regulate cell fate decisions such as myoblast differentiation into myotubules[1]. Current methods to recapitulate the endogenous conductivity of human tissues employ electrically responsive polymers. However, the majority of conductive biomaterials present cells with a two-dimensional (2D) environment[2] that does not accurately capture their native three-dimensional, (3D) anisotropic environments. Moreover, healthy skeletal muscle is defined by its 3D microenvironment, composed of aligned collagen fiber bundles[3]. Here we developed a conductive 3D collagen-based scaffold with a highly aligned, anisotropic pore structure for muscle tissue engineering.

Methods: Conductive collagen scaffolds were fabricated by directional lyophilization of a suspension of type I collagen, chondroitin sulfate, and polyprrole (PPy) using a custom designed mold (*Fig. 1A)*. PPy microparticles were synthesized via an oxidation reaction with $FeCl_3$ and mixed directly into the collagen suspension prior to lyophilization. The conductivity of the scaffolds was analyzed via chronopotentiometry, while mechanical properties were characterized by oscillatory shear rheology. Pore microstructure was assessed using ImageJ and MATLAB. Immortalized mouse myoblasts (C2C12s) were used for all experiments. C2C12s were cultured within scaffolds containing varying weight percentages of PPy (0.1, 0.2, 0.5) and cell viability was assessed using AlamarBlue. Cell growth and spreading were determined by confocal imaging of the scaffold backbone and cytoskeletal F-actin staining.

Results: Conductive PPy powder doped with $FeCl_3$ was homogenously distributed into a suspension of collagen and chondroitin sulfate in acetic acid. Directional heat transfer during lyophilization resulted in scaffolds containing a highly aligned and homogeneous 3D pore microstructure with an average pore size of 155 ± 27 µm in the transverse plane and 218 ± 49 µm in the longitudinal plane. The open pore architecture allows for cell infiltration and migration. Conductivity of the scaffolds was modulated by incorporating varying amounts of PPy powder into the collagen suspension. The addition of 0.5 wt.% PPy resulted in a five-fold increase in conductivity (1.42 ± 0.18 mS/m) when compared to the collagen scaffold control (0.27 ± 0.04 mS/m) (*Fig. 1B*). Additionally, PPy-doped collagen scaffolds displayed a similar viscoelastic regime when compared to collagen controls (viscoelastic limit of ~ 1% strain) and a comparable storage modulus of ~ 10 kPa, similar to moduli previously shown to be beneficial for muscle tissue engineering[4,5]. C2C12 mouse myoblasts cultured over one week within 0.5 wt.% PPy-collagen scaffolds showed sustained, increasing metabolic activity (*Fig. 1C*). This was not statistically different when compared to the collagen only control. Confocal imaging indicated improved cell alignment and cytoskeletal extension along the collagen backbone (*Fig. 1D, E*) simulating the cytoskeletal organization of healthy skeletal muscle.

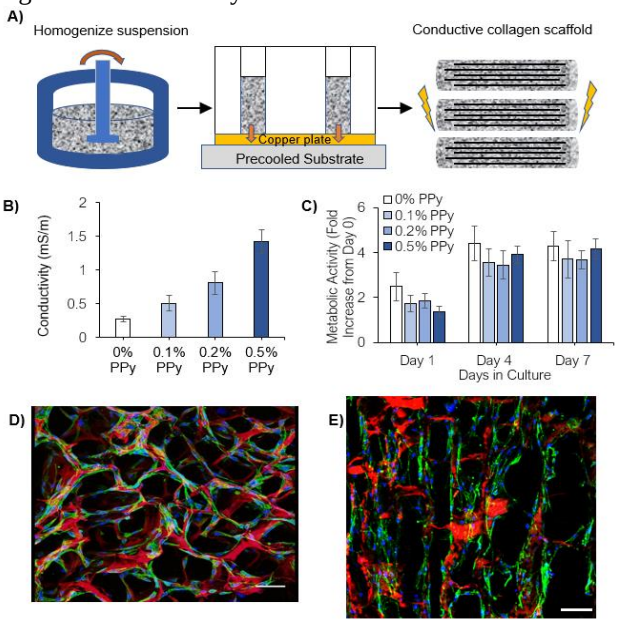

Figure 1. A) Schematic of PPy-collagen scaffolds fabrication. B) Chronopotentiometry shows increasing conductivity with increasing wt.% PPy (n = 3) C) AlamarBlue data shows that PPy scaffolds supported sustained, increasing metabolic activity D) Representative images of C2C12s (nuclei (*blue*), F-actin (*green*)) in PPy-collagen scaffolds (collagen (*red*)) transverse and E) longitudinal plane respectively show cells spreading within scaffolds and conforming to scaffold microstructural contact guidance cues. Scale bars: 100 µm.

Conclusions: We developed a highly aligned, 3D, conductive collagen scaffold via directional lyophilization of PPy-doped collagen suspension for skeletal muscle tissue engineering. Scaffold anisotropy facilitated improved cytoskeletal organization along the pore microstructure similar to healthy muscle. The addition of 0.5 wt.% PPy did not alter the mechanics of the scaffold nor reduce C2C12 viability. Ongoing work aims to explore the differentiation of C2C12 cells and subsequent maturation of myotubules within the scaffolds.

References: 1) Jun I, *Biomaterials*, 2009, 11: 2038-47; 2) Balint R, *Acta Biomaterialia*, 2014, 10: 2341-53; 3) Gilles A, *Muscle Nerve*, 2011, 44: 318-31; 5) Engler A, *Cell*, 2006 126: 677-89; 6) Gilbert P, *Science*, 2010 329: 1078-81;