Multi-Layered Electrospun Polydioxanone Collagen Blend Matrices

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Statement of Purpose: Nanofibrous matrices have shown considerable potential in tissue regeneration, and electrospinning is a relatively easy and inexpensive method for fabricating nanofibrous matrices. Electrospun matrices mimic the extracellular matrix in terms of fiber dimension and mechanical properties which can influence cell morphology, proliferation, and differentiation. Synthetic polymers have been electrospun by many groups to develop partially biomimicking scaffolds in terms of fiber dimension [1-3] and mechanical properties [4-5], but there is also interest in using natural biopolymers for electrospinning applications because, in addition to providing optimal fiber dimension and mechanical properties, many natural materials contain biofunctional molecules that also aid in triggering cell attachment, proliferation, and differentiation [6]. Here we report the development of matrices composed of synthetic and natural electrospun materials. To manipulate their mechanical properties, these matrices were fabricated with either random fibers alone, aligned fibers alone, and with both random and aligned fibers, which was achieved by layering individual electrospun sheets.

Methods: Multilayered matrices were electrospun from polydioxanone (PDO)/collagen Type I blend solutions. Matrices with aligned and random fibers were spun by varying the speed of collector rotation. To fabricate a multilayered construct, the initial layer of electrospun matrix was first laid down on a smooth polyethylene sheet and wetted with 100% ethanol, ensuring a flat sheet. Second and third layers were laid down on top of the first one. The multilayer structure was then allowed to dry. Specimens were immersed in phosphate buffered saline at room temperature for 30 minutes before mechanical evaluations. Uniaxial tensile testing was performed using a 50 N load cell with a crosshead speed of 20 mm/min on an Instron uniaxial tensile testing machine with a 2.5 cm gage length and 1.2x5 cm samples. Suture retention strength was determined on 10x20 mm samples. One end of the sample was fixed to the stage clamp of the uniaxial tensile testing machine and the opposite end was connected to the other clamp by 5-0 prolene suture material, which was placed through the electrospun sample, 2 mm from its edge. The distance between the stage clamps was 2 cm and crosshead speed was 125 mm/min.

The cytotoxicity of PDS/collagen Type I electrospun matrices was evaluated based on a procedure adapted from the ISO10993-5 standard test method [7]. Cell culture medium consisted of Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% L-glutamine (DMEM Complete). DMEM Complete was conditioned by incubating in the presence of electrospun matrices for 48 hours. DMEM Complete alone was incubated for 48 hours as a negative control. The metabolic activity of human dermal fibroblasts incubated in electrospun conditioned media and in control media were evaluated using alamar blue. Human umbilical artery smooth muscle cells (UASMC) were used to evaluate the initial stage of *in vitro* cellular interactions with multilayer matrices. Cellular response evaluations were conducted on flat sheets of aligned and randomly aligned matrices since cells are exposed on either aligned or randomly aligned fibrous surface when seeded on the multilayer matrices. Metabolic activity for both aligned electrospun and random electrospun matrix cultures were evaluated using alamar blue. Matrix morphology and cell distribution on the scaffolds were characterized using scanning electron microscopy. For statistical analysis, data sets from different matrix groups were screened by one-way ANOVA and pairwise comparisons were performed using Tukey's honestly significant difference test. For all statistical analysis differences were considered significant at $P < 0.05$.

Results: Mechanical properties including stress at failure, strain at failure, Young's modulus, and suture retention strength of various matrix configurations were compared. Our data suggest that aligned fibers oriented perpendicular to the direction of pull increases suture retention and reduces tensile strength. Suture strength ranged from 0.75 N to 1.86 N for aligned fibers oriented parallel and perpendicular to the direction of pull, respectively. The tensile strength was 1.31 MPa for fibers aligned perpendicular and 3.11 MPa for fibers aligned parallel to the direction of pull. Other constructs comprising layered sheets of random and aligned fibers in various orientations had intermediate tensile and suture retention strengths, suggesting that these parameters are tunable. Collagen Type I and PDO showed no adverse effects in *in vitro* cytotoxicity tests, and in culture with HDFs and UASMCs, matrices with aligned fibers and random fibers were comparable with respect to cellular metabolic activity, attachment, and proliferation.

Conclusions: The results show that it is possible to fine-tune the mechanical properties of electrospun matrices by layering sheets of aligned fibers, random fibers, or both. Combining material properties with the multilayering technique described in this study can further improve application-dependent scaffold performance.

References:

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