Decellularized Lung Extracellular Matrix Electrospun with Poly-L-Lactic Acid for Tissue Engineering

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Statement of Purpose: Decellularized extracellular matrix is a well-established strategy for creating a macroscopic and micro-structure appropriate scaffolding with tissue-appropriate adhesive ligands for directing cell growth and differentiation. Electrospinning allows for customized macroscopic and microscopic properties useful for both tissue engineering and physiologic testing. In this research, decellularized pig lung extracellular matrix (PLECM) has been combined with Poly-L-Lactic Acid (PLLA) in electrospinning material fabrication, with concentration-dependent changes in mechanical properties. Biocompatibility of this biologic scaffold has been assessed to determine the benefits of native tissue ECM proteins for cell attachment and epithelial transition. This material has applications for regenerative lung-resection patches, and research mimicking ECM in vitro with tissue culture or advanced lung physiology models such as microfluidics and bioreactors.

Methods: Pig lung (donated from Smithfield Hams) was decellularized by an established protocol (England et. al., 2010) using detergents and ionic solution, lyophilized at -80° C, then freezer milled using SPEX 6700 to a fine powder. 140 mg and 70 mg of powdered PLECM were combined with 3 mL Hexafluoro-2-propanol and mixed for 24 hours on a vortexer. 400 mg PLLA in 1 mL of HFP was mixed for the same period of time. The PLECM solution was poured into a syringe and pushed through 150x150 mesh count stainless steel type 304 wire cloth into the PLLA solution and vortexed again for an hour to create the final PLLA/PLECM/HFP solution. Solutions with 35 mg, 17.5 mg, and 0 mg PLECM with 100 mg PLLA per mL HFP were tested.

Table 1.

[PLECM]	Flow Rate	Voltage	Distance
35 mg/mL	4 mL/hr	27 kV	27 cm
17.5 mg/mL	4 mL/hr	27 kV	27 cm
0 mg/mL	4 mL/hr	15 kV	27 cm

3 mL of PLECM solution were electrospun at the parameters in table 1, which were arrived at by the establishment of a stable Taylor cone. The collector was a 7.6 x 2.5 x 0.6 cm stainless steel plate rotating at 600 RPM and translated 14 cm across the horizontal plane at 30 translations per minute.

Material properties were determined using MTS Bionix 200 with TestWorks 4.0 Software. Scanning Electron Microscopy (SEM) images were taken using a JEOL LV-56-10 at 20 kV after carbon sputter-coating. Statistical analysis using one-way ANOVA w/ Tukey multiple comparisons test. Cell Proliferation of human small airway epithelial cells (SAEC, Lonza) on the various concentrations of PLECM previously stated were determined with a Picogreen DNA florescence assay 48 hours after seeding. Epithelial gene expression of Vimentin and E-cadherin from SAECs on electrospun material was determined using quantitative PCR (qPCR). Immunostaining of scaffolds seeded with small airway epithelial cells for one week was performed to assess morphology and cell-cell connections using e-cadherin (Cell Signaling) and alpha smooth muscle actin (Sigma) antibodies and a Zeiss LSM 710 Laser Scanning Microscope

Results: Increasing concentration of the PLECM decreased the elastic modulus of the electrospun scaffold (Fig 1).

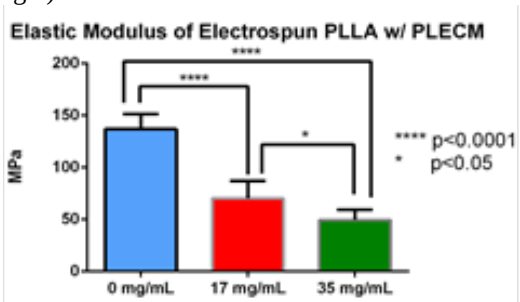

Figure 1.

Uniform fibers 400-600 nm diameter with minimal debris from the dissolved PLECM are visualized with SEM (Fig 2). Increased PLECM content significantly increased the cellular proliferation, showing the most significant increase in 35 mg/ml PLECM. PLECM content increased the e-cadherin gene expression concludes increased cell-cell adhesion. Vimentin expression significantly decreased with the presence of PLECM, showing less epithelial-to-mesenchymal transition as compared to the control (100 mg/ml PLLA).

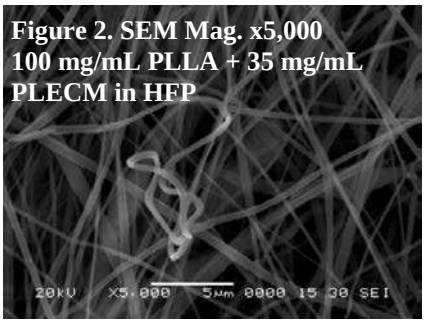

Figure 2. SEM Mag. x5,000 100 mg/mL PLLA + 35 mg/mL PLECM in HFP

Conclusions: This material is promising for use in tissue engineering. The addition of gelatin controls is underway to show the importance of all ECM proteins other than just collagen derivatives in the success of this scaffold. Further gene expression quantification will be done to confirm the presence of epithelial specific markers. Future goals include the formation of Bronchiole like constructs for more specialized applications.

References: (England, K. A. Development of a Decellularized Lung Bioreactor System for Bioengineering the Lung : The Matrix Reloaded, Tissue Engineering Part A. 2010:16:2581-2591)

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