Extracellular Matrix Hydrogels from Decellularized Lung Tissue

Robert A. Pouliot, Nabil S. Mikhaiel, Ben Wong, Rebecca L. Heise, PhD.
Virginia Commonwealth University

Statement of Purpose:

Diseases categorized as chronic lower respiratory disease are the third leading cause of death in the US, resulting in almost 140,000 deaths in 2010 [1]. Treatment strategies for lung pathologies are limited by the complexity and adverse conditions of the lung. Unique challenges include: (1) the complex architecture of interweaving airways, vasculature, and functional alveolar structures, (2) a volatile mechanical environment (respiration), and (3) multiple inherent mechanisms for clearing agents from the tissue. To address these challenges we have developed a thermosensitive extracellular matrix (ECM) hydrogel from decellularized lung tissue which contains many of the growth factors, binding sites, integrins, and other proteins involved in tissue specific cell-matrix recognition. ECM hydrogels have been developed from an increasing number of tissues, including: cardiac, epidermis, bladder, articular cartilage, nerves, and adipose sources [2-7]. "Injectability," allows these materials to reach and settle into a defect before undergoing self-assembly at 37°C. This mechanism would encapsulate any cells or drugs sequestered in the pre-gel solution. The goal of this research is to evaluate the potential for this material to address treatment challenges in the lung.

Methods:

Tissue decellularization and processing strategy were adapted [7,8] and have been optimized for tissue isolated from porcine lung. Decellularization was accomplished using a dual injection strategy, perfusing the airways and vasculature with solutions including: 0.1 % Triton-X100, 2% sodium deoxycholate, DNase, and sodium chloride. Histology and SEM imaging were performed on both intact and decellularized tissues for comparison. Rheometery was used to assess the mechanical properties of the hydrogels. Sample modulus was recorded as the temperature was increased to 37°C to induce gelation. Fully formed gels were subjected to a frequency sweep to further characterize the gel mechanics. Turbidity assays were also run to further examine gelation kinetics. Mass spectrometry was performed to identify specific protein components found in the pregel solution. The hydrogel degradation profile was determined by measuring the protein released over time using a Peirce BCA protein assay. Picogreen assays were used to determine human mesenchamal stem cell (hMSC, Rooster Bio) attachment and viability.

Results:

A qualitative study comparing intact porcine lung tissue slices to decellularized lung tissue slices shows that the decellularization process preserves many of the structures of the lung (Fig. 1). Rheological measurements show that the self-assembly mechanism of the pre-gel solution start around 35 °C and that the majority of the mechanical change related to the gelation occur shortly after the gel reaches physiologic body temperature of 37°C(Fig 2. A). Gelation behavior was confirmed in a turbidity experiment. Mass spectrometry confirmed presence of various forms of collagen (types I,II and VI), keratin (types I and II), proteoglycans, fibronectin, laminin, and fibrillin in the pregel solution. The majority of the protein degradation and release form the hydrogels was found to occur in the first 72 hours following gelation. Human mesenchymal stem cells were found to have a significantly increased rate of attachment/adherence to ECM coated plates over collagen coated and TC plastic controls (Fig 2,B).

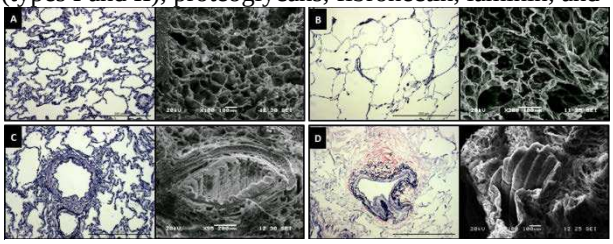

Figure 1. A. Intact lung parenchyma B. Decellularized lung parenchyma C. Intact airways D. Decellularized airways

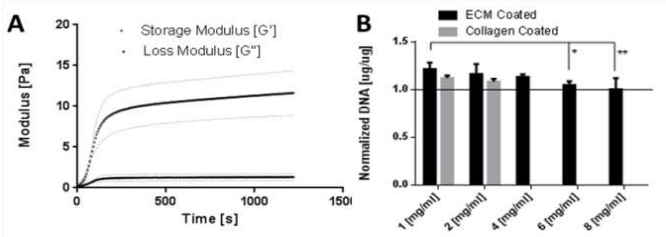

Figure 2. A. Gel Rheometry of 8mg/mL ECM hydrogel B. Cell attachment assay after 2 hours of hMSC

Conclusions: This study has successfully demonstrated that decellularized lung tissue can be processed and digested to create a thermo-sensitive material that behaves as a liquid at room temperature, but rapidly self assembles into a hydrogel as the environment approaches 37 °. The composition, structure, gelation kinetics, mechanical properties, and protein release degradation behavior of the material have been characterized. In addition we have conducted initial in vitro experiments which suggest that naturally derived ECM proteins can increase cell attachment and maintain the viability of encapsulated cells. The results of this study support continued investigation lung derived hydrogels.

References:

[1] (Kochanek KD. Natl. Vitl. Stat. Rep. 2011:59:1)
[2] (Singelyn JM. J. Am. Coll. Cardiol. 2012;59:751–63)
[3] (Kwon JS. Int. J. Pharm. 2013;454:183–91)
[4] (Wolf MT. Biomat. 2012;33:7028–38.)
[5] (Young DA. Acta. Biomat. 2011;7:1040–9)
[6] (Medberry CJ. Biomat. 2013;34:1033–40)
[7] (Freytes DO. Biomat. 2008;29:1630–7.)
[8] (Price AP. Tis. Eng. Prt. 2010;16:2581–91)

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