Investigating Photo-Initiator Cytotoxicity in a ECM-Polymer Composite Substrate with Tunable Mechanical Properties

Ethan John1, Kaseya Xia2, Alison Kahn2,3, Jessica Peura4, Robert Pouliot2, and Daniel Weiss2

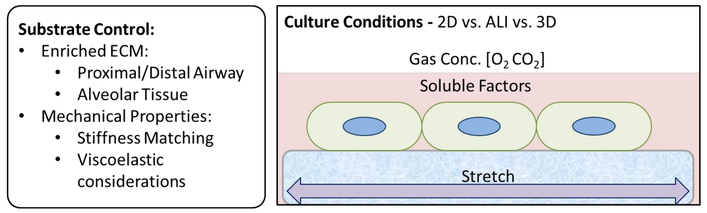
[1]Department of Medicine, University of Vermont, Burlington, Vermont, USA, [2]Biomedical Engineering Department, Rochester Institute of Technology, Rochester, New York, USA,

Background

Decellularized organs and extracellular matrix proteins (ECM) have increasingly been used in the development of artificial organs and novel biomaterials. The use of decellularized ECM protein with the goal of 3D bioprinting tissue will contribute to the development of artificial, whole organs. Due to the gradient of structural-mechanical properties along lung airways from trachea to bronchi and alveoli, a printable substrate with tunable stiffness is required to mimic specific sections of lung tissue. The purpose of this work was to identify a photo-crosslinkable hydrogel containing decellularized porcine lung ECM and methacrylated alginate that can support human derived lung derived epithelial cells and mesenchymal stromal cells. The cytotoxicity of Eosin Y/NVP/TEAO was compared that of LAP to determine the feasibility of each photo initiator system.

Target Applications

Advanced Tissue Modes and Culture Systems



Fabrication/Bioprinting Scaffolds for Tissue Engineering

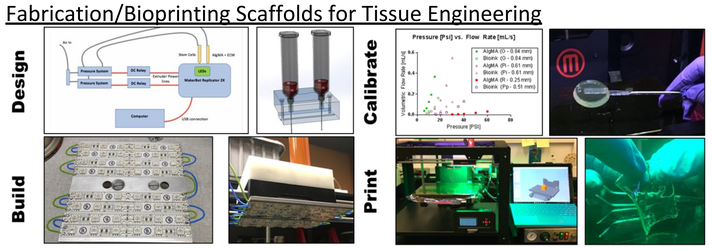


Figure 1: Target applications for design and development of polymer /ECM materials includes (1) materials for engineering cell culture substrates and (2) as bioinks for 3D fabrication of tissue engineering scaffolds

Methods/Materials

Materials:

- Decellularized Porcine/Human Lung ECM Powder (Bulk or Structure Enriched)
- Polymers for pilot Studies: Alginate Methacrylate, Chondroitin Sulfate Methacrylate, Gelatin Methacrylate, and Hyaluronic Acid Methacrylate
- Photo-Initiator Systems: TEA/EY/NVP or LAP (Biokey)
- Green (532 nm) and UV (365 nm) LEDs for photocrosslinking

Methods:

Pig lungs were decellularized according to a previously developed protocol which used a constant-flow based perfusion pump to sequentially inoculate airways and vasculature with deionized H2O, hypertonic saline, triton X-100, sodium deoxycholate (SDC) and DNAse. Bulk samples (with large airways removed), were lyophilized, LN2 milled into a fine powder, and then digested at 10 mg/mL in pepsin digest solution containing 1 mg/mL pepsin (Sigma aldrich) in 0.01 M HCl for 16 hours. Digested protein was neutralized on ice, and the lyophilized. Solubilized ECM lyophilate was resuspended to achieve a higher protein concentration 15 mg/mL), one of two photo initiators (Eosin Y/NVP/TEAO or LAP (Lithium phenyl-2,4,6-trimethylbenzoylphosphinate)) and different concentrations of methacrylated alginate (3%, 2%, 1%) to generate substrates of increasing stiffness (~13.5kPa – 105 kPa). The combined materials were incubated for 30 minutes to allow ECM self-assembly and then photocrosslinked for 5 minutes with green light (for Eosin Y PI) or blue light (for LAP PI). A549 and human mesenchymal stromal cells were seeded at 10k cells/well on top of each substrate. AlamarBlue with 3-hour incubation was used to assess growth at 1 and 4 days. Live stain was used to make transmitted light images which showed morphology after 4 days.

Physical Behavior and Mechanical Testing

Protein Self-Assembly vs. Polymer Photocrosslinking

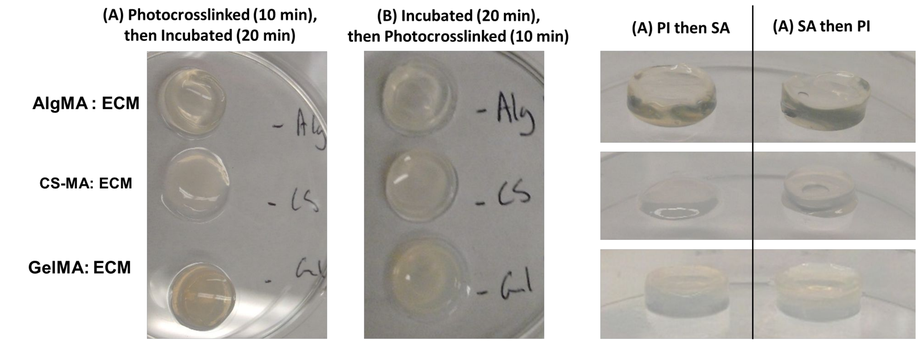


Figure 3: Qualitative observation of several Polymer/ECM systems suggests that photocrosslinkning the materials first may hinder ECM self-assembly later. (4 mg/mL ECM, 1.5% AlgMA, CS-MA, GelMA)

Photo-initiator Cytotoxicity

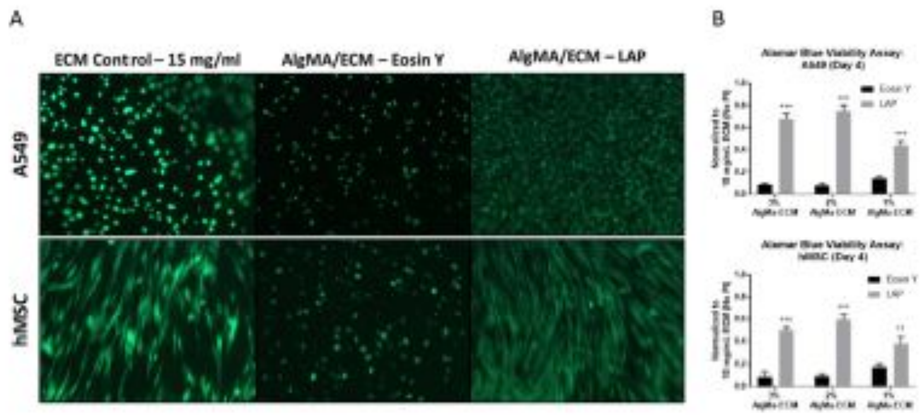


Figure 3: (A) Live Dead results comparing A549 and MSC morphology when cultured on polymer/ECM composite substrates photocrosslinked with Eosin Y or LAP photoinitiation systems compared to ECM only without any photoinitiator, and (B) Alamar Blue Viability data collected from the same wells shows that the LAP PI system is much less toxic than the Eosin Y based system.

Increasing Stiffness vie Polymer Concentration

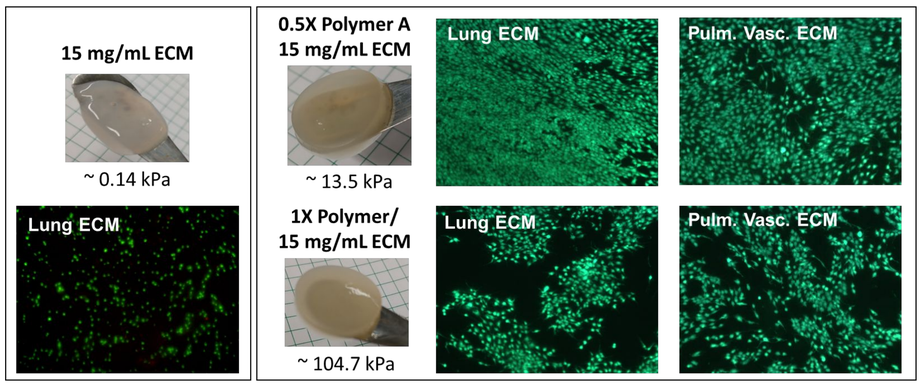
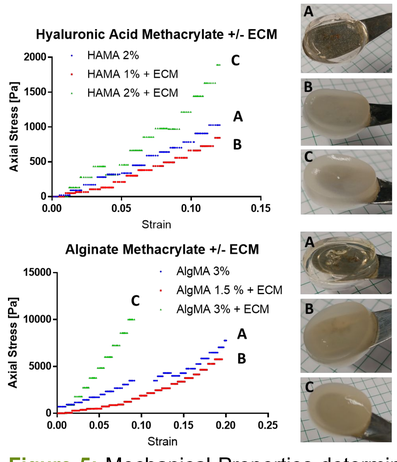


Figure 6: Calcien AM staining of C10 (alveolar epithelial cells) grown on ECM/polymer substrates for several days. While all substrates were able to support cell attachment and viability for several days we have not yet concluded any substrates stiffness or ECM source contributions to cell behavior.

Physical Behavior and Mechanical Testing



Sample	Young's Modulus (kPa)
ECM (15 mg/mL)	0.14
3% AlgMA	61.5
1.5% AlgMA + ECM	13.5
3% AlgMA + ECM	104.7
2% HaMA	7.7
1% HaMA + ECM	3.5
2% HaMA + ECM	15.0

Figure 5: Mechanical Properties determined via compression of fully formed gels, these gels where allowed to self assemble in the incubator for 30 minutes and then photocrosslinked for 10 minutes. These results confirm that addition of ECM to the polymer solution increases stiffness.

Tunable Substrate Mechanics Effect Cell Behavior/Viability

Figure 6: New data – almost complete

Conclusions

- Mechanically tunable ECM-polymer substrates support cell attachment and proliferation significantly better when photocrosslinked using LAP instead of EY photo-initiation system. We are using this tunable polymer-ECM substrate system to isolate the effects of stiffness and ECM source in isolation. Future studies will be conducted using relevant cell types on matrix isolated from decellularized, dissected and enriched, alveolar, small airway, and large airway structures at a range of stiffnesses.

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