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

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Introduction:

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.

Materials and 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 H_2O, hypertonic saline, triton X-100, sodium deoxycholate (SDC) and DNAse. Bulk samples (with large airways removed), were lyophilized, LN_2 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.

Results and Discussion:

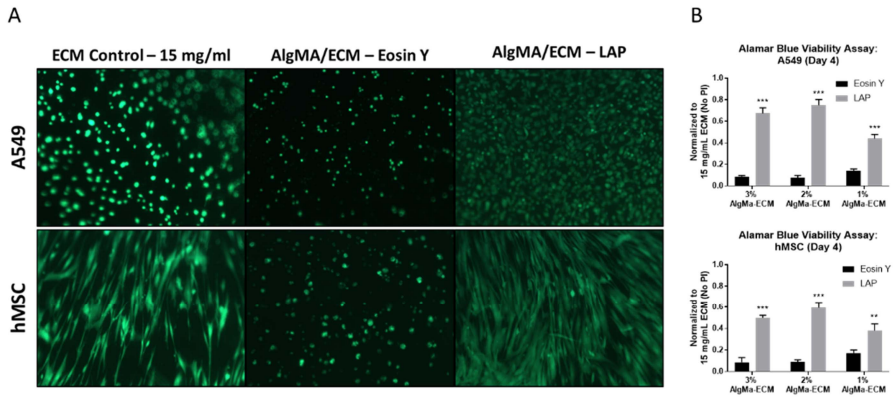

Figure 1: (A) Live Dead results comparing A549 and MSC morphology when cultured on polymer/ECM composite substrates photocrosslinked with Eosin Y or LAP photoinitiation systems compared to ECM only without any photoinitiator, and (B) Alamar Blue Viability data collected from the same wells shows that the LAP PI system is much less toxic than the Eosin Y based system.

The results of the Live/Dead staining and AlamarBlue experiment are shown in figure 1. Live dead results show that both the A549 and hMSC cultures were inhibited on the substrates crosslinked with the Eosin Y system but able to attach and proliferate on those crosslinked using LAP. Previously, we have determined that substrates crosslinked with the Eosin Y PI system at this concentration can be rinsed several times over 24 hours to reduce cytotoxicity for long term viable cell culture. The AlamarBlue results were normalized to the ECM only control wells and confirm the LAP crosslinked substrates supported significantly higher cell viability. The Live/Dead results seem to show more viable cells on the AlgMA/ECM substrates compared to the ECM only substrates which is inconsistent with the AlamarBlue results. It is possible that the LAP interacts with the assay to produce this effect, but additional investigation is needed.

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.