Methacrylated Alginate for Use as an Adherent Pulmonary Pleura

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Statement of Purpose: Lung transplantation remains the sole treatment option for many end stage lung diseases. In addition to a critical shortage of donor lungs, transplantation efficacy is largely susceptible to acute and chronic rejection and complications from required immunosuppressive medications. One potential strategy is the utilization of de-cellularized whole lungs as scaffolds for seeding with autologous cells, obtained from eventual transplant recipients, to grow functional lung tissue *ex vivo*. The development of innovative techniques to dissect out multiple small lung segments (1-3cm^3), which retain their 3-dimensional structure, from individual cadaveric or donor lungs unsuitable for transplant could be used to develop high-throughput studies and accelerate progress. However, one of the consequences of de-cellularization and segmentation is a damaged pulmonary pleura. This has severely limited the use of segments for research. Therefore, we sought to synthesize an adherent artificial pleura providing a physical exterior barrier for de-cellularized human lung segments allowing for re-cellularization and mechanical ventilation.

Methods: Methacrylated alginate (Alg-MA) was synthesized utilizing an anhydrous reaction which allowed for controlled degrees of polymer modification. Briefly, high molecular weight alginate (Alg) with a high number of guluronic acid residues (Manugel GMB, FMC Biomedical) was rendered soluble in an organic solvent through an ion exchange with an ammonium salt (hexadecyltrimethylammonium bromide). A 1% (w/v) Alg in DMSO solution was reacted with methacrylic anhydride and 4-dimethylaminopyridine (catalyst) for 24 hours at room temperature. The mixture was then precipitated and washed with ethanol, and subsequently hydrolyzed though pH adjustment to neutral in deionized water. Aqueous solutions of Alg-MA were mixed with eosin Y (photosensitizer), triethanolamine (photoinitiator) and 1-vinylpyrrolidone (catalyst). Methacrylation was verified with ^{1}H-NMR. Exposure of solutions to green light (510nm) using a series of green LEDs resulted in crosslinking to form hydrogels. Viscosity and gelation of the Alg-MA were investigated using and AR2000 series rheometer (TA Instruments). Utilizing a 40 mm cone (1°) geometry and a 27 µm gap distance, continuous flow ramps (viscosity) and gelation time sweeps (10% strain, 510nm light) were performed. Cytocompatibility was assessed on 8% (w/v) Alg-MA gels using an MTT assay. Thin coatings of Alg-MA were applied and crosslinked on decellularized lung segments. Small airways were cannulated and segments were ventilated to assess the mechanical stability of the Alg-MA coating. Cellular retention was assessed following airway or vascular inoculation into excised segments.

Results: Methacrylation of Alg was successful (DOM ≈65%, Fig 1b). The resulting Alg-MA was found not to be cytotoxic, as compared to both positive and negative (EtOH) controls (Fig 1a). Viscosity was found to be lower as compared to the starting material (Fig 2). Degradation may have occurred as a result of the modification process, leading to decreased viscosities. Gel points (non-terminal) were determined to be 4.5-10 minutes. Thin coatings of Alg-MA were successfully crosslinked on decellularized lung segments and segments could be ventilated. Alg-MA coated segments were found to both permit cell inoculation through either the airways or vasculature and enhance their retention as compared to uncoated segments.

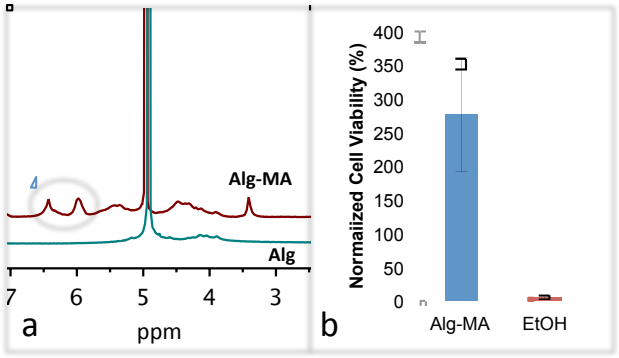

Fig. 1: a)*Normalized hMSC viability after 24 hours culture.* ***b)*** *^{1}H-NMR spectra showing methacrylate peaks.*

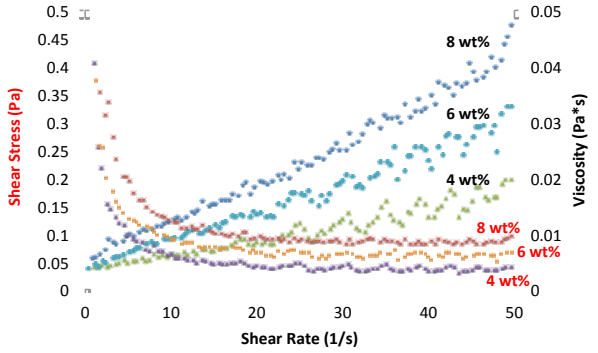

Fig. 2: *Viscosity/Shear Stress curves from continous flow ramp testing.*

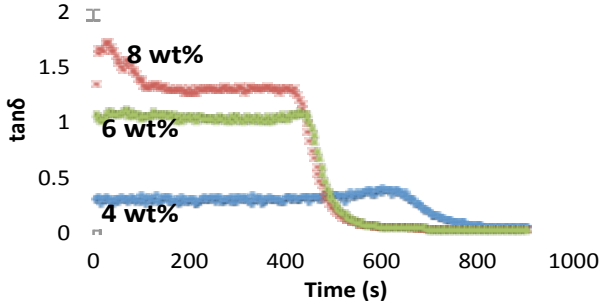

Fig. 3: *tanδ curves during photocrosslinking showing gel points of each wt% Alg-MA solution.*