Design and Synthesis of an Adherent Artificial Pulmonary Pleura

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Statement of Purpose: Recent developments in tissue engineering utilizing decellularized whole organs as scaffolds and advances in stem/progenitor cell biology have offered the potential of using cadaveric lungs recellularized with appropriate cells for *ex vivo* lung regeneration. However, optimal techniques for regenerating lung *ex vivo* are not clear. Further, while preliminary successes have been achieved using decellularized whole rat lungs which have been recellularized and implanted, there have been no reported successes with whole or partial transplantations using human lung tissue. One of the current limiting factors with decellularized lungs is that the pleura can be disrupted, allowing both leak of inoculated cells and air during ventilation. Therefore, we sought to design and synthesize an adherent artificial pleura providing a physical barrier for decellularized cadaveric human lungs which is cytocompatible, biodegradable, anti-adhesive to cells, and mechanically stable, permitting airway and vasculature inflation.

Methods: Normal and emphysematous human lungs were obtained from the autopsy services at Fletcher Allen Hospital in Burlington, Vermont. These lungs were de-cellularized using a previously published protocol of intratracheal and vascular perfusion using sequential incubations with Triton X-100, sodium deoxycholate, sodium chloride, and DNase. Pieces of de-cellularized lungs were then coated with a 2.5% sodium alginate solution or left uncovered. The artificial pleura consisted of a calcium alginate hydrogel [1] generated by crosslinking the sodium alginate-covered lung pieces with 3% calcium chloride solution. Segments were then inoculated through the airways with either human lung fibroblasts, or through the vasculature with endothelial progenitor cells and then statically cultured and harvested at various time points. Presence of the artificial pleura, cell engraftment and survival were assessed by histochemical and immunohistochemical staining. *In vitro* anti-adhesion assays were performed using human alveolar carcinoma cells (A549s). The integrity of the artificial pleura and its ability to be inflated was assessed through instillation of Trypan-blue through both the vasculature and airway branches in each piece.

Results: De-cellularized human lung segments which had been encased in the artificial pleura were found to retain up to 75% of the inoculated cells while those segments devoid of pleura only retained about 5%. The artificial pleura was found to be intact and lining the periphery of the de-cellularized matrix by histological inspection. The lungs pieces were able to be fully inflated and retained the Trypan blue without rupture of the artificial pleura. A549s did not adhere to the artificial pleura material during the *in vitro* anti-adhesion assay.

Fig 1. Human lung piece after inflation with Trypan blue demonstrating the ability of the artificial pleura to maintain airway and vasculature integrity and mechanical stability for inflation.

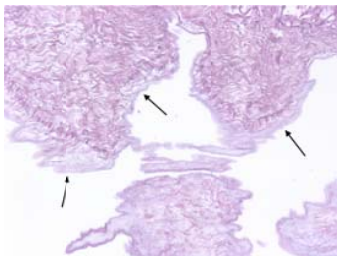

Fig. 2 Histological assessment of artificial pleura confirms that it has adhered to the outside periphery of the de-cellularized human lung segment. (arrows)

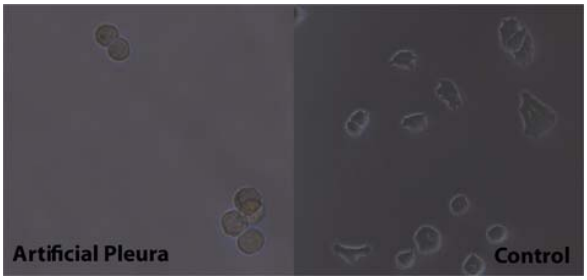

Fig 3. The artificial pleura prevents cellular adhesion of A549s. The cells can be seen to be attaching to the tissue culture dishes (controls) and adopting an adherent phenotype while those cells seeded on the artificial pleura remain spherical and are not adherent

Conclusions: A method and material for a mechanically stable, adherent artificial pulmonary pleura was achieved through a two-step process of coating de-cellularized human lung segments with sodium alginate and crosslinking with calcium chloride. The artificial pleura retained inoculated cells significantly better than the de-cellularized control (devoid of the artificial pleura) and was able to be inflated through the airways and vasculature. A549s were nonadherent to artificial pleura.

References

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