

modulates cell phenotype and associated behavior, and by extension tissue/organ health and disease. The present study evaluated the ability of an orally administered hydrogel, derived from healthy porcine esophageal ECM, to mitigate the dysplastic and metaplastic changes in the esophageal epithelium that results from reflux.

***Methodology:** The Levrat model, in which a surgical transposition of the duodenum to the distal esophagus occurs, was used as the rodent model for this study. After 24 weeks of disease development, the surviving nineteen (19) rats were evaluated and showed pathology ranging from dysplasia to adenocarcinoma. These 19 rats were then divided into three (3) treatment groups (n=6-7/group): buffer control, eECM hydrogel, or heterologous urinary bladder matrix (UBM-ECM) hydrogel; receiving a twice-daily oral dose of their respective treatment; for 21 days. Endoscopic footage was reviewed and analyzed by blinded clinicians.

***Results:** Results revealed a disease state reversion in five of the seven animals treated with eECM and in five of the six UBM-ECM treated animals. There was no marked disease improvement in two of the seven eECM treated and one of the six UBM-ECM treated animals. Untreated animal controls displayed no disease improvement in four of the six animals, while two animal controls did experience some improvement to disease state. Microscopic evaluation following necropsy showed a reduction in Muc2+ and Muc5AC+ goblet cells in eECM treated animals compared to the pepsin control animals.

***Conclusion/Significance:** Results of the present study support previous findings of the effect of normal ECM upon altered cell morphology, tissue disease/health, and have therapeutic implications for esophageal disease and a variety of other pathologic conditions.

B 38 - Decellularised Pleural Membrane Patches In Pulmonary Regenerative Medicine

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***Purpose/Objectives:** Prolonged alveolar air leaks as post-surgical complications to routine lung resections and biopsies are a significant cause for patient morbidity. Extended duration of chest tube drainage and emergency revision surgeries are the standard approaches for its clinical management. Transplantable decellularised pleural membrane patches as adjuncts to traditional intraoperative closure techniques could reinforce the mechanical barrier, reducing the incidence and severity of sustained air leaks. As a treatment modality, it can provide the relevant physiological cues that stimulate endogenous tissue regeneration. Adopting the classic tissue engineering paradigm of conditioning cells in a bio-instructive microenvironment niche, we focused on optimising a decellularisation protocol for porcine pleural membranes (PPM) and characterising the biological scaffolds for their retention of the innate mechanical strength, biochemical composition, microarchitecture, and bioactivity of the native pleural membranes.

***Methodology:** PPM decellularisation was carried out using a combinatorial approach of physical (freeze-thaw cycles) and chemical (0.5% sodium deoxycholate and 1% Triton-X 100 in 10mMTris buffer) treatments. Protocol efficiency was determined with histological analysis (Hematoxylin & Eosin, Alcian blue, and Picrosirius red staining), nuclear membrane integrity study (DAPI staining), and quantitative bioassays (Picogreen assay for nuclear DNA quantification, Sircol™ insoluble collagen assay, and dimethylmethylene blue (DMMB) glycosaminoglycan assay). Decellularised PPM were also assessed for their cytotoxicity (Live-Dead cytotoxicity kit, Invitrogen™, and Trypan blue exclusion assay) and biocompatibility (MeT-5A cell-line seeding and culture).

***Results:** H&E staining of decellularised PPM showed the absence of stained nuclei, consistent with the significant reduction ($p < 0.0001$) in DAPI stained nuclei counts against native controls. PicoGreen assay confirmed efficient decellularisation as the quantified nuclear DNA in the decellularised PPM was less than 50 ng/mg. of dry weight of tissue. Staining for sulphated glycosaminoglycans (sGAG) and collagen with alcian blue and picosirius red respectively, exhibited minimal disruption to the innate structural alignment of the native ECM fibers. However, quantifying collagen and GAG content per mg. of dry weight of tissue, in the decellularised PPM showed a significant reduction in comparison with the native controls. Mechanical characterisation studies revealed a significant increase in decellularised membrane thickness but not affecting the innate membrane stiffness as the estimated Young's modulus in the decellularised PPM ($12782.7 \text{ kPa} \pm 3874$) was comparable with the native controls ($9259.5 \text{ kPa} \pm 2079$). *In vitro* cytotoxicity assay carried out on seeded MeT-5A cells in contact with decellularised PPM for five days, exhibited minimal effect on cell proliferation and viability. Preliminary scaffold biocompatibility studies revealed promising results with the decellularised PPM seeded with MeT-5A cells promoting cellular attachment, proliferation, and viability for two weeks under standard culture conditions.

***Conclusion/Significance:** Our pilot study represents a step forward in deriving bioactive ECM scaffolds in the form of decellularised PPM. Future work entails expanding the characterisation regime to include proteomics and ultrastructural studies. Studying the recellularisation dynamics of the cell-seeded scaffolds using primary mesothelial cultures will underpin our research towards developing proof of concept for the application of the relatively unexplored decellularised pleural membranes in biological scaffold-based therapeutic approaches.

B 40 - 3d Scaffolds From Agglomeration Of Collagen-gelatin Particles Associated With A Plant Extract Of Aloe Vera: Design, Elaboration And Physicochemical Characterization And In Vitro Biological Performance.

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***Purpose/Objectives:** Agglomeration of polymeric particles has been applied to produce scaffolds with improved mechanical properties that can be used as delivery systems for bioactive molecules. Sintering by heat or binders are commonly used to compact polymeric particles. However, high temperatures may denature natural polymers, and binding agents reduce the porosity of the resulting scaffolds. Collagen I and gelatin - its derivative - are biomaterials widely used for tissue engineering applications because their biocompatibility, low cost, and availability. For this reason, this work developed and characterized collagen I microgels (MGs), agglomerated microgels (MGAs), and scaffolds of agglomerated particles (APs).

***Methodology:** MGs were obtained by emulsification-gelation, and the effect of three different concentrations of collagen (3, 2.5 and 1% w/w) and homogenization methods (magnetic stirring, shear stress, and ultrasound) on the size and size distribution of the MGs was studied. MGAs manufactured by agglomerating MGs using gravitational force (1000 to 3000 xg), were dehydrated and lyophilized to obtain scaffolds of agglomerated particles (APs). MGs, MGAs, and APs microstructural (pore size and porosity), and mechanical properties (storage module G' , loss modulus G'' and Young's modulus) were