Carbonyldiimidazole Immobilization of an Extracellular Matrix Protein Mixture onto poly(HEMA) and Decellularized Esophageal Tissue

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Statement of Purpose: Decellularized tissues have recently been used as replacements for defects in the gastrointestinal tract [1]. Our lab has developed a decellularization protocol for rat esophageal tissue. The protocol is currently being modified for the pig. The process creates a natural biomaterial that is comprised mainly of the extracellular matrix (ECM) proteins left behind after the removal of cellular material. The basement membrane is the extremely thin, dense layer of protein directly adjacent to the epithelium in the esophageal ECM consisting mainly of laminin and collagen IV. The bulk of the ECM beneath the basement membrane is comprised of collagen I. The retention of the basement membrane in the decellularized tissue is thought to be necessary for effective reepithelialization. In the porcine esophagus, the extraction methods can be damaging to the basement membrane. A procedure for restoring critical basement membrane proteins would be desirable. Carbonyldiimidazole (CDI) immobilization chemistry has previously proven effective for the covalent attachment of protein onto a surface with hydroxyl reactive groups [2]. The purpose of this project is to use CDI chemistry to attach extracellular matrix proteins to the surface of the decellularized tissue thus artificially recreating a temporary basement membrane. This will be done using single protein solutions as well as the ECM protein mixture, Matrigel® (mainly laminin and collagen IV).

Methods: The substrates used in this work will be the decellularized tissue created in the lab from porcine esophagus and poly(HEMA) spin cast on glass coverslips. The *in vitro* cell culture work uses rat esophageal epithelial cells (REEC). CDI (Sigma) immobilization chemistry was applied to the poly(HEMA) cast coverslips using laminin, collagen I, and Matrigel® (all proteins purchased from BD). Electron spectroscopy for chemical analysis (ESCA) was used on the poly(HEMA) cast coverslips in order to confirm the immobilization of protein to the surface. A REEC adhesion assay and an alamarBlue™ (Biosource) proliferation assay have been used (on the coverslips) and compared to the adhesion and proliferation of REEC on a thin film of Matrigel®. Time of flight secondary ion mass spectroscopy (ToF-SIMS) analysis will be used to compare spectra from laminin and collagen. Following the poly(HEMA) work, CDI chemistry will then be applied to the decellularized esophageal tissue. Scanning electron microscope images will show the surface characteristics of the decellularized tissue before and after the immobilization. Tissues will also be analyzed using immunochemistry to see cross section architecture and specific ECM protein location.

Results / Discussion: ESCA analysis was done on the spin cast poly(HEMA) coverslips which had gone through the CDI immobilization to covalently link proteins to the surface. The composition of nitrogen on the surface from the ESCA spectra was used to confirm the immobilization chemistry and give an initial estimate of differences between the three conditions. The laminin and Matrigel® had close to 7% nitrogen composition while the collagen I had a 3% nitrogen composition. An adhesion assay was performed using REECs. The following table shows REEC adhesion over 24 hours.

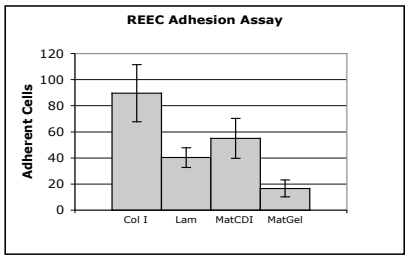

Figure 1: REEC adhesion assay on CDI bound collagen I, laminin, and Matrigel® as well as gelled Matrigel®.

Conclusions: The ESCA data shows values that are consistent with the desired surface chemistries. It would be expected that the nitrogen compositions would be similar for laminin and Matrigel® because Matrigel® is comprised mainly of laminin. The REEC adhesion assay shows that cells adhere in higher numbers to the CDI immobilized Matrigel® and laminin surfaces compared to the gelled Matrigel®. Thus, CDI immobilization of Matrigel® leads to a more attractive substrate for REEC growth than gelled Matrigel®. Adhesion was highest yet on collagen I. This might be expected because collagen I is the major ECM component underneath the basement membrane. In a wound healing situation, it might be necessary for epithelial cells to attach quickly to the underlying ECM collagen and immediately start to remodel it with their own basement membrane proteins. This would explain the quick adhesion, but further tests will be done in order to determine the long term effects of these immobilized proteins. Future ToF-SIMS analysis will give insight into actual fragments of protein left on the surface after immobilization. The idea of immobilizing a mixture of proteins using CDI chemistry is valuable by itself, but the conjunction of that technique with the decellularizing process should lead to an exciting new material.

References:

1. Badylak, S. F., et al., J Surg Res. 2005 Sep;**128**(1):87-97.
2. Martin, S.M., et al., J Biomed Mater Res A, 2004. **70**(1):10-9.

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