Preparation and Characterization of Porcine Esophageal Extracellular Matrix

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Statement of Purpose: Biologic scaffolds composed of extracellular matrix (ECM) are commonly used to facilitate a constructive remodeling response in several types of tissue, including the esophagus [1-3]. In both preclinical and clinical studies, constructive and functional remodeling of injured esophagi has resulted following surgical placement of an ECM scaffold derived from either urinary bladder (UBM) or small intestinal submucosa (SIS-ECM). Recent work has described potential benefits of ECM derived from homologous tissue (i.e., site-specific ECM) compared to heterologous tissue in certain anatomic locations [4, 5]. It is currently unknown whether site-specific ECM may be beneficial for esophageal reconstruction.

The objective of the study was to prepare and characterize ECM derived from porcine esophagus (eECM). Esophagi were collected and decellularized by a method sufficient to meet stringent decellularization criteria [6]. Biochemical and mechanical properties of the ECM were then characterized by a number of quantitative and qualitative measures. Lastly, scaffold compatibility was assessed by an in-vitro cell viability assay and an in-vivo rat model.

Methods: Esophagi were harvested from market weight pigs and split longitudinally. The mucosa and submucosa were isolated by mechanical separation from the muscularis propria and scraped to remove the squamous epithelium. The remaining tissue, composed primarily of the lamina propria, muscularis mucosa, and submucosa, was then subjected to a series of agitated detergent and enzymatic baths. The eECM scaffold was then lyophilized and sterilized by ethylene oxide. After paraffin embedding, immunohistochemistry labeling was performed for common ECM basement membrane and non-basemenet membrane proteins including collagen IV, laminin, and fibronectin. Cell viability assays were performed using multi-laminate devices. Additional colorimetric and ELISA-based assays for collagen, glycosaminoglycans, and growth factors were performed after mechanical comminution. Viability of perivascular stem cells (PVSCs) was assessed in-vitro using the Live/Dead assay (Invitrogen) after exposure to the scaffold for 48h. The host response to implantation of an esophageal ECM scaffold was assessed in a previously described partial thickness rat abdominal wall defect.

Results: After decellularization, the eECM scaffold contained no intact nuclei as evidenced by staining with hematoxylin and eosin and DAPI. Remnant DNA concentration following processing was 48 ± 12.9 ng/mg dry weight of material. In addition, no intact DNA was detected in the eECM samples following decellularization and any remaining fragments were less than 200 base pairs. Quantification of s-GAGs via Blyscan™ assay showed that eECM (4.5 ± 0.8 mg/g) contained similar amounts of s-GAGs after decellularization as native (3.8 ± 0.5 mg/g). Growth factors were measured by ELISA-based assays. While vascular endothelial growth factor (VEGF) was not found at detectable levels in eECM, basic fibroblast growth factor (b-FGF) remained at a concentration of 1127.4 ± 779 ng/g.

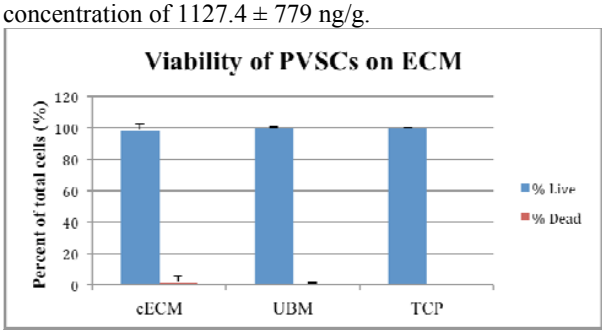

Figure 1. In-vitro viability of PVSCs on eECM

Cytocompatibility assessment of the eECM scaffold in-vitro (Fig 1) showed no statistical difference in viability of PVSCs between eECM, UBM, and tissue culture plastic (TCP). When implanted in an abdominal wall defect in the rat, histologic analysis showed an early dense mononucelar cell infiltrate at 14 days post-operation accompanied by neo-matrix deposition with the presence of vasculature throughout the material and rapid degradation. By 35 days, the site was composed of organized connective tissue and islands of skeletal muscle at the periphery. Histomorphologic score, based upon previously described criteria [7], was determined by three blinded investigators to be 11.4 out of a possible 15; a score indicative of excellent host compatibility.

Conclusions: This study shows that ECM derived from porcine esophageal tissue is compliant with established criteria for decellularization. This ECM was found to preserve many of the structural components as well as bioactive molecules that are necessary to sustain cellular activity and promote tissue repair. The scaffold is cytocompatible in-vitro and biocompatible in-vivo. Animals treated with eECM scaffolds had high histomorphologic scores and the observed host response was consistent with early stages of constructive remodeling. Further study is required to determine the efficacy of an eECM scaffold in the esophageal location and to determine if site-specific ECM may be preferable for esophageal reconstruction.

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