

# Bi-Layer Silk Fibroin Grafts Support Functional Tissue Regeneration in a Rat Model of Onlay Esophagoplasty

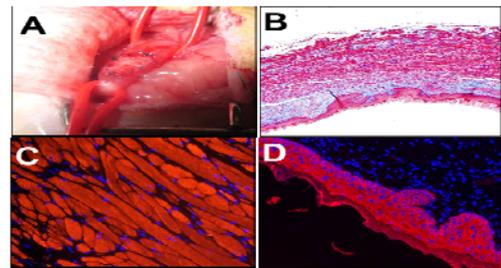
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**Statement of Purpose:** Long-gap esophageal defects resulting from esophageal atresia, tracheoesophageal fistula and Barrett's esophagus, are often repaired with gastric pull-up or interposition grafts using either jejunum or colon [1]. Unfortunately, these approaches are associated with severe adverse complications such as esophageal dysmotility and dysphagia [2]. Decellularized tissue grafts, such as small intestinal submucosa (SIS), have been investigated as alternatives for esophageal construction, however limitations including graft contracture and incomplete tissue regeneration have been observed [3]. We hypothesized that a biodegradable, bi-layer implant derived from *Bombyx mori* silk fibroin (SF) would serve as a superior option for esophageal tissue repair. The unique scaffold configuration consists of a porous SF foam which promotes host tissue ingrowth while an annealed SF film functions to provide a fluid-tight seal for retention of hollow organ contents [4]. In this study, we compared the performance of bi-layer SF grafts with conventional SIS biomaterials in a rat model of onlay esophagoplasty.

**Methods:** Bi-layer SF matrices were fabricated from aqueous SF solutions by a solvent-casting/salt leaching process in combination with film casting as previously described [4]. SIS matrices (4-ply) were obtained from Cook, Bloomington, IN. Sterile scaffold groups (Bi-layer SF: N=22; SIS: N=22) were evaluated for up to 2 m of implantation (graft size, 21 mm<sup>2</sup>) in an onlay esophagoplasty model using female Sprague-Dawley rats (6-8 wks of age) following IACUC-approved protocols. Sham controls (N=20) receiving esophagotomy alone were performed in parallel. Animal weight was monitored every wk prior to scheduled euthanasia. At 2 m post-surgery, all groups were assessed for the formation of functional tissues by micro-computed tomography ( $\mu$ CT), ex vivo contractility, histological, immunohistochemical (IHC), and histomorphometric evaluations. Data were analyzed with the Kruskal-Wallis test and post-hoc Scheffé's method. Statistically significant values were defined as  $p < 0.05$ . **Results:** Prior to scheduled euthanasia, rats implanted with bi-layer SF and SIS scaffolds both displayed survival rates of 91% in comparison a 100% survival rate for esophagotomy controls. Animals in each experimental group were capable of solid food consumption following a 3 d liquid diet and demonstrated similar degrees of weight gain throughout the 2 m study period. End-point  $\mu$ CT analysis of both scaffold groups revealed preservation of organ continuity similar to control features with no evidence of contrast extravasation, fistulas, dilatation, or strictures. Gross tissue evaluations at 2 m post-op demonstrated

extensive host tissue ingrowth spanning the entire area of the original implantation site in both implant groups. Longitudinal contraction was noted in 50% of the SIS grafts following harvest with a 43-79% reduction in original graft length. In contrast, 10% of bi-layer SF implants were contracted with an 11-21% reduction in original graft length. Similar levels of ex vivo contractile forces were generated in the consolidated tissues supported by both scaffold groups in response to electrical field stimulation and KCl. At 2 m post-op, Masson's trichrome staining (MTS) demonstrated the presence of chronic inflammatory reactions and severe fibrosis at sites of SIS implantation. In contrast, only scant areas of mild fibrosis were observed in the SF group. SIS and bi-layer SF scaffolds underwent extensive degradation over the course of the study period wherein only minute residual fragments were noted within consolidated tissues. Histomorphometric analyses revealed that both matrix groups promoted the formation of similar levels of pan-cytokeratin+ epithelia in comparison to controls. In contrast, bi-layer SF scaffolds supported significantly higher degrees of myosin+ skeletal muscle formation (4-fold) as well as synaptophysin 38+ synaptic boutons (4-fold) within regenerated tissues in respect to the levels achieved with SIS grafts.



**Figure 1.** [A] Rat onlay esophagoplasty with bi-layer SF scaffold. [B] MTS of regenerated esophageal tissue supported by SF graft at 2 m post-op (cross-sectional view, 5X). [C] IHC of skeletal myosin (Cy3, red) in regenerated muscularis externa from [B]. [D] IHC of pan-cytokeratin (Cy3, red) in de novo esophageal epithelium from [B]. Nuclear stain (blue).

**Conclusions:** Bi-layer SF scaffolds support functional tissue regeneration of esophageal defects. These matrices support significantly higher degrees of innervation and skeletal muscle formation, reduced graft contraction, and lower extents of fibrosis in comparison to SIS grafts.

**References:** [1] Shen KR. J Thorac Cardiovasc Surg. 2008; 135:1185-1187. [2] Romeo C. J Pediatr Surg. 2000; 35:740-7. [3] Totonelli G. World J Gastroenterol. 2012; 18:6900-7. [4] Seth A. Biomaterials. 2013; 34:4758-6.