Comparison of Tissue Engineered Intestine Produced from Non-woven Polyglycolic Acid, CollaTape Collagen, and Nanofiber Poly (Ɛ-caprolactone) Scaffolds

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Statement of Purpose: Short bowel syndrome (SBS) is a major clinical problem affecting newborns, children and adults. Patients with SBS require total parenteral nutrition to survive, which is extremely costly and is associated with many complications. Small bowel transplantation is an option, but is limited by donor availability and complications of immunosuppression. Our approach to this unsolved medical problem is the production of tissue-engineered intestine (TEI). The purpose of the current studies was to compare tissue engineered intestine produced from non-woven polyglycolic scid (PGA), CollaTape Collagen, and nanofiber Poly (Ɛ-caprolactone) (PCL) scaffolds, and to provide valuable information on the required scaffold properties for producing TEI.

Methods: PGA Biofelt (2 mm thickness and 60 mg/cm^3 density) was purchased from Biomedical Structures (Warwick, Rhode Island) and CollaTape collagen from Zimmer Dental (Warsaw, Indiana). Tubular scaffolds were prepared by wrapping the above materials around stainless steel mandrels to produce 1.0 cm length × 4.7 mm diameter tubes. The tubes were coated with 5% polylactic acid (PLLA; Sigma, St. Louis, MO) in chloroform (Fisher Scientific, Pittsburgh, PA). Once the solvent was completely evaporated, the scaffolds were soaked in 100% ethanol for 30 min and then washed 3 times with PBS. Scaffolds then underwent an additional 30 minute coating with 0.4 mg/ml collagen type I (Advanced BioMatrix, San Diego, CA) and were sterilized with ethylene oxide.

To prepare tubular PCL nanofiber scaffolds, a 5% solution of PCL (Mw 65,000, Sigma-Aldrich, St. Louis, MO) in 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP, Sigma-Aldrich) was prepared, placed in a 60 ml syringe with a 20 gauge blunt tip needle, and electrospun using a high voltage DC power supply (Glassman High Voltage, Inc., High Bridge, NJ) set to +16 kV, a 20 cm tip-to-substrate distance and a 5 ml/hr flow rate. Salt crystals previously sieved to sizes between 90 and 106 µm were introduced into the Taylor Cone and the falling fiber beneath it. The electrospinning was stopped when the desired wall thickness (500 µm) was achieved. The salt crystals were leached out by exposure to DI water. The scaffolds were soaked in 70% ethanol for 30 minutes and rinsed with PBS prior to use.

Intestines harvested from Lewis rat pups were pooled, minced into ~5-7 mm pieces, digested as described by Sala, et al,[1] and concentrated crypts were obtained as follows. The mixed cells were filtered respectively with 200, 70, and 25 µm sieves. The cells harvested from the 25-70 µm fraction contained concentrated intact crypts as proven by separate experiments, and were suspended in DMEM containing 10% premium select fetal bovine serum at 3 × 10^6 /ml. The different scaffolds were seeded with 200 µl of the cell suspension and sutured to the inside surface of the abdominal wall of the dam of the pups. Twelve samples from each material were harvested after four weeks of *in vivo* incubation and examined histologically.

Results: The morphology of TEI produced from non-woven PGA scaffolds demonstrated a mucin-filled open lumen (panel A) with the intestinal architecture containing long villi, dense crypts, and abundant goblet cells (panel D). The morphology of TEI produced from CollaTape collagen had a mucin-filled lumen (panel B), but the intestinal architecture demonstrated narrow villi on the smooth muscle-like surface with absence of crypts (panel E). The morphology of TEI produced from nanofiber PCL demonstrated a collapsed lumen (panel C), with some mucosal cysts at the edge of the scaffolds that were filled with non-resorbed scaffold remnants (panel F).

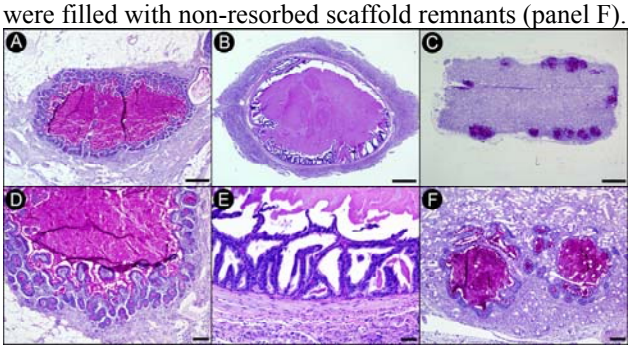

Figure 1. Histology of TEI produced from crypt-seeded PGA scaffold (A and D), CollaTape scaffold (B and E), and nanofiber PCL scaffold (C and F). PAS staining, scale bar: 800 µm in A, B, and C; 200 µm in D, E, and F.

Conclusions: We conclude that scaffolds for the production of TEI should meet several criteria. First, the scaffolds should have an appropriate mechanical strength to keep the lumen open during the period of *in vivo* incubation or the collapsed lumen will prevent the implanted cells from obtaining nutrients from the host, as demonstrated by the nanofiber PCL scaffolds. Second, the scaffolds should have an appropriate porous structure to allow crypts to extend beneath the villi, as demonstrated by the CollaTape collagen scaffolds. Third, the scaffolds should have an appropriate degradation rate to minimize fibrous tissue formation on the side wall of the TEI, as demonstrated in the nanofiber PCL scaffold in which the non-resorbed PCL material significantly increased the side wall thickness in TEI. In conclusion, among the three tested scaffolds in this study, non-woven PGA scaffolds are suitable for growing TEI. Additional modifications are needed to produce superior scaffolds for TEI production.

References:1. Sala, FG, Tissue Eng Part A. 2011;17: 1841-1850.