Evaluation of Biomaterials for Esophagus or Small Intestine Tissue Engineering

Toyin Knight, Richard Payne, Joydeep Basu, John W. Ludlow, Namrata Sangha, Kelly Guthrie,
Manuel Jayo, Elias Rivera, Deepak Jain.
Tengion, Inc., Winston-Salem, North Carolina, USA

Statement of Purpose: Current treatment strategies for patients needing esophageal or small intestine (SI) tissue replacements are often associated with adverse effects, which negatively affect quality of life. This study seeks to apply tissue engineering principles to the regeneration of these organs. Previously, these principles have been successfully used to develop implantable cell/biomaterial composites for reconstructing bladder, another tubular organ with laminar wall architecture. In these cases, de novo organogenesis was catalyzed following implantation of the composite (aka, construct) and resulted in the regeneration of a functional organ[1-4].

Methods: Biomaterials of different forms and composition were evaluated. Poly-caprolactone (PCL) foams of pore sizes 23-300µm were made by a solvent cast–particulate leached method as well as polyglycolide (PGA) fibers in various forms coated with poly-DL-lactide-co-glycolide (PLGA). These included coated PGA nonwoven mesh (PGAnw), woven mesh (PGAw) and braided tube (PGAb). Smooth muscle cells were expanded *ex vivo* from rat visceral adipose (Ad-SMC) and used to seed biomaterials for *in vitro* and *in vivo* evaluation[2]. Assessment of this cell-biomaterial interaction *in vitro* was by live/dead staining, cell attachment/proliferation assay (MTS) and scanning electron microscopy (SEM). For evaluation of esophageal and SI regeneration *in vivo*, PGAw and PGAnw were trimmed to 5mm x 4mm rectangular patches and seeded with Ad-SMC to make constructs. PGAb with Ad-SMC was used to make tubular SI constructs. Patch constructs for both esophagus and SI were sutured with non-resorbable suture over a rectangular defect of approximately 5mm x 4mm that was cut into the tissue wall to expose the lumen in adult rats. Tubular SI constructs (10mm length, 4mm I.D.) were used to connect anterior and distal portions of the SI after transverse dissection. Omentum was sutured over the constructs to provide a source of vascularization. Animals were euthanized at time points ranging from 6 days to 20 weeks post-implantation. At necropsy, tissues were harvested, fixed in formalin and paraffin embedded for sectioning and staining with Trichome. The non-resorbable suture marking the defect site allowed comparison of the native and the regenerated tissue.

Results: *In vitro* assays showed all materials had acceptable cell viability, proliferation, and morphology (Figure 1). Lower cell viability and proliferation were seen on the smaller-pore PCL foams. *In vivo*, sectioning through the defect sites of the PGAnw esophagus patch construct at 10 weeks post-implant (Figure 2) the PGAw SI patch construct at 16 weeks post-implant (Figure 3), and PGAb SI tubular construct at 20 weeks post-implant (data not presented) showed complete re-epithelialization of the luminal mucosal surface and a submucosa with partial regeneration of the muscularis externa. There was no evidence of remnant scaffold fibers, calcification, necrosis or bacterial colonization.

Figure 1.Left: Live/Dead staining of rat Ad-SMC on PCL foam, 150-250µm pore size, 10X. Right: SEM images of rat Ad-SMC on PGAnw, 170X

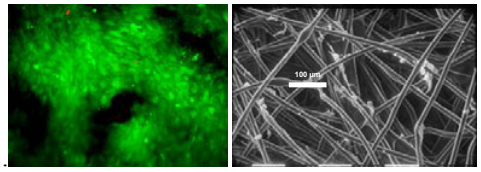


Figure 2. Regenerated esophageal tissue from PGAnw patch construct.

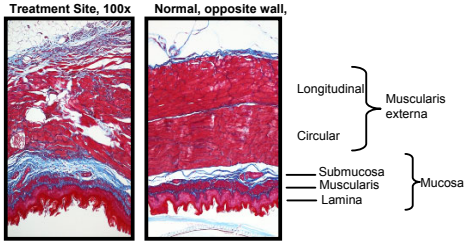
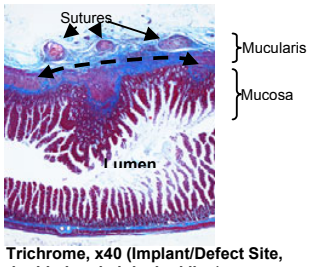


Figure 3. Regenerated SI tissue from PGAw patch construct.



Trichrome, x40 (Implant/Defect Site, double-headed dashed line)

Conclusions:

- PGA and PCL biomaterials showed biocompatibility with Ad-SMC *in vitro*. PGA materials were suitable for making esophageal and SI patches and SI tubular constructs.
- *In vivo* implantation of PGA patch constructs resulted in esophageal and SI tissue regeneration within 10 and 16 weeks, respectively.
- *In vivo* implantation of PGAb tubular constructs resulted in SI tissue regeneration within 20 weeks.

Acknowledgements: We thank Kim Mihalko (Carolinas Medical Center) for animal surgeries.

References:

1. Basu J. Trends Biotechnol. 2010;28(10):526-33
2. Basu J. *7th Annual ISSCR Meeting*, 2009.
3. Jayo MJ. Regen Med 2008;3:671
4. Joseph D. J Urol 2009; 181:555