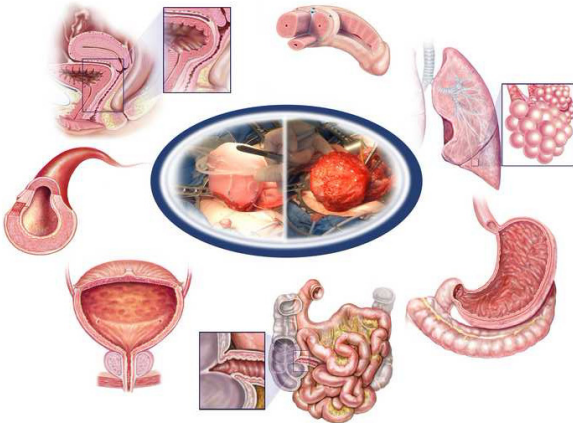


## Introduction:

The demand for esophageal tissue replacement is greatest in pediatric patients presenting with congenital long-gap esophageal atresia, since direct anastomosis is not a treatment option. The most common cause for esophageal replacement in adults is surgical resection of the esophagus due to cancer. Standard of care for both patient groups uses esophageal lengthening techniques, substitution with intestinal tissue, or transposition of the stomach; treatments that are frequently associated with post-operative complications and a reduction in quality of life. Tissue engineering principles have been successfully used in developing implantable cell/biomaterial composites for reconstructing tubular organs with laminar wall architecture (e.g., bladder) where *de novo* organogenesis is catalyzed following implantation of the composite (aka, construct) and has resulted in the regeneration of a functional neo-organ.<sup>1-4</sup> (Figure 1) The esophagus represents a specialized iterative variation of a laminarily organized architecture. The current study demonstrates, in principle, the application of this organ regeneration technology platform towards regeneration of components of the gastro-intestinal tract, in particular, the esophagus.

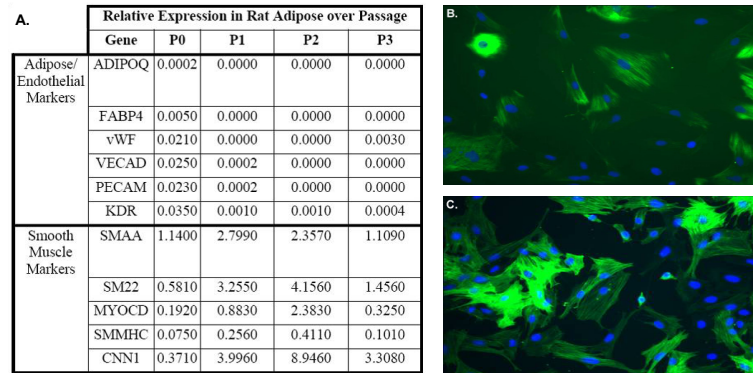
**Figure 1.** The bladder is a foundational platform for tubular organ regeneration. (Center, left) Implantation of Tengion's Neo-Bladder Augment (NBA) at the dome of a native bladder during augmentation cystoplasty of a patient presenting with neurogenic bladder secondary to spina bifida. (Center, right) Wrapping of omentum around NBA for vascularization. Examples of laminarily organized tubular organs that may be regenerated using the foundational platform technology demonstrated for the bladder. Top, clockwise: penis, lung, esophagus and stomach, small intestine, bladder, vessel, vagina.



## Materials and Methods:

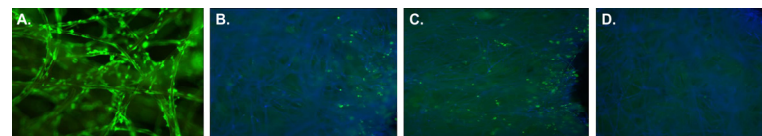
Adipose-derived smooth muscle cells (Ad-SMC) were expanded *ex vivo* from male Lewis rat visceral adipose as described for porcine cells<sup>3</sup> and used to populate biomaterials for esophageal implantation. Woven or nonwoven meshes based on polyglycolide and polylactide were used to assemble esophageal patches. Rectangular biomaterial patches of approximately 5 mm x 4 mm were seeded directly with Ad-SMC (passage 2) to form constructs, which were matured for 5 days prior to implantation in female Lewis rats. Esophageal wall defects (5 mm x 4 mm) that completely exposed the lumen were made and then patched with a construct that was secured by non-resorbable suture. Omentum was sutured over the patch to provide a source of vascularization and water tightness. Animals were euthanized humanely at multiple time periods ranging from 6 days to 20 weeks post-implantation. Esophagi were harvested, fixed in formalin, and embedded in paraffin prior to sectioning and staining with Masson's Trichrome. The non-resorbable suture marking the defect site facilitated comparison of native esophagus and neo-esophagus tissues to evaluate the regeneration of the native esophageal wall structure comprised of laminar layers of mucosa and muscle.

**Figure 2.** Phenotypic confirmation that Ad-SMC are expanded from rat visceral adipose



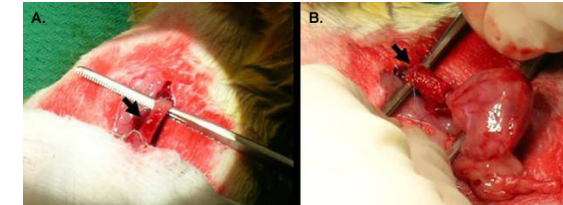
- Gene expression of adipogenic markers adiponectin (ADIPOQ) and fatty-acid binding protein-4 (FABP4), endothelial markers von Willebrand factor (vWF), cadherin-5 (VECAD), platelet/endothelial cell adhesion molecule (PECAM), and kinase insertion domain receptor (KDR), and smooth muscle markers smooth muscle  $\alpha$ -actin (SMAA), transgelin (SM22), myocardin (MYOCD), smooth muscle myosin heavy chain (SMMHC), and calponin (CNN1) were assessed by qRT-PCR (A)
- In initial isolate cultures (P0), marker expression characteristic of multiple cell types is expected based on the cellular composition of adipose tissue; however, adipocyte and endothelial marker expression decreases markedly upon the first passage and becomes barely detectable in subsequent passages, supporting the conclusion that these cell types fail to expand in these cultures.
- In contrast, expression of smooth muscle markers persists across multiple passages (P1-P3 in A).
- CNN1 (B) and SMAA (C) were chosen for analysis by immunofluorescence at passage 2 based on our experience that these proteins are the most reliably detected in statically cultured SMC.
- Taken together, these data support the conclusion that we have successfully isolated and cultured smooth muscle cells from rat adipose tissue (Ad-SMC).

**Figure 3.** Scaffolds are biocompatible with rat Ad-SMC and constructs support endothelial migration



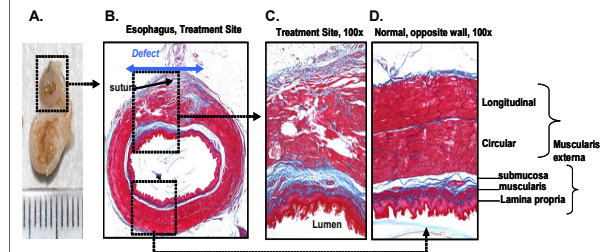
- Viable Ad-SMC (passage 2) are seen covering the scaffold matrix at 7-days post-seeding (A) by Live/Dead staining (green fluorescence = live cells), indicating that the scaffold biomaterial supports rat Ad-SMC attachment and viability
- To evaluate the potential for endothelial migration into esophageal patches, esophageal tissue was inserted into cell-free scaffolds or Ad-SMC seeded constructs and endothelial cytochrome visualized by immunofluorescence 8 days after tissue insertion.
- Cytochrome staining in cell-free scaffolds was observed primarily in the vicinity of the tissue insertion site, with little endothelial migration into the biomaterial (B).
- Conversely, cytochrome staining was widely distributed beyond the site of tissue insertion in Ad-SMC seeded constructs (C), indicating that more esophageal endothelial cells migrated from the tissue into the construct matrix.
- As expected, no cytochrome staining was observed in control constructs where esophageal tissue was not inserted (D).

**Figure 4.** Surgical procedure for making esophageal defects and applying patches



- Esophagus was brought into workable field by distension. The shape of the defect after surgical resection was somewhat oval in shape as a result (A).
- Patched esophagus showing the non-absorbable sutures used to secure the implant at the edges so they could be used to delineate the boundaries of the regenerated esophageal tissue after tissue was harvested and prepped for histology (B).

**Figure 5.** Regeneration of esophageal tissue at 10 weeks post-implantation



- A continuous section of the esophagus, containing intact native tissue anterior and posterior to the implant site, was removed at necropsy for histological assessment (A).
- Cross section of the implant site and the opposing native intact tissue stained with Masson's Trichrome (B).
- Higher magnification of the implant site (C) and opposing wall of the intact native esophagus (D) revealed complete re-epithelialization of luminal mucosal surface and submucosa in the implant site. The muscularis externa of this site shows evidence of regeneration consisting of fibrovascular connective tissue and smooth muscle cells.
- Taken together, these data suggest that, by 10 weeks after implantation of an Ad-SMC/biomaterial construct similar to that used to regenerate urinary tissue, neo-esophagus tissue regeneration has occurred, as characterized by the formation of all three esophageal wall layers; mucosa, muscularis, and serosa.

## Conclusions:

- Regenerating tubular urinary neo-organs using cell/biomaterial composites has been demonstrated in both human and animal studies, from which a foundation platform using SMC/biomaterial composites has emerged.<sup>1-4</sup>
- The regeneration of native-like esophageal tissue following implantation of an Ad-SMC/biomaterial composite similar to that used to regenerate urinary tissue<sup>2</sup>, as presented here, demonstrates the potential to extend Tengion's foundational organ regeneration platform beyond the urinary tract to regenerate other tubular organs.

## References:

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