Visceral Hollow Organ Reconstruction: Preclinical Experience with Silk Fibroin Grafts

The Good, the Bad, and the Translational

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Overview of Visceral Hollow Organs

- Organs of the Digestive, Respiratory, and Urogenital Tracts
- Provide Storage and Transport Functions for Body Systems
- Tubular or Elliptical Organs with Specialized Epithelium Lining Muscular or Cartilaginous Walls.

Epithelia

Muscle

Bladder

Trachea

α-SMA

α-SMA MYH
Bi-Layer Silk Fibroin (BLSF) Grafts for Reconstruction of Visceral Hollow Organs

Silkworm Cocoons

Silk Processing and Graft Fabrication

Bi-Layer Silk Fibroin Graft

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Bladder Diseases and Consequences

**Neurogenic Bladder**
- Spina Bifida, 0.7 per 1000 births;
- Spinal Cord Injury, ~250,000 in US

**Bladder/Cloacal Exstrophy**
- 1 per 50,000 births

**Posterior Urethral Valves**
- 1 per 5,000 male births

**Severe Voiding Dysfunction**
- BPH/LUTS, ~35 million in the US

Exstrophy

Spina Bifida

Normal System

Posterior Urethral Valves (PUV)

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Enterocystoplasty: Current Standard of Care

*Complications*: chronic urinary tract infections, stones, metabolic abnormalities, bowel dysfunction, malignancy
Limitations in Acellular and Cell Seeded Grafts for Bladder Augmentation: Clinical Trials

Autologous Cell Seeded Biodegradable Scaffold for Augmentation Cystoplasty: Phase II Study in Children and Adolescents with Spina Bifida

David B. Joseph,* Joseph G. Boror, Roger E. De Filippo,† Steve J. Hodges‡ and Gordon A. McLorie

Conclusions: Our autologous cell seeded biodegradable scaffold did not improve bladder compliance or capacity, and our serious adverse events surpassed an acceptable safety standard.

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Bladder augmentation with small intestinal submucosa leads to unsatisfactory long-term results

M. Schaefer a,*, A. Kaiser b, M. Stehr a, H.J. Beyer a

Conclusion: Bladder augmentation with SIS in humans failed to fulfill the hopes raised by animal studies. Due to the insufficient increase in bladder compliance and therefore failure to accomplish sufficient protection of the upper urinary tract, bladder augmentation with SIS cannot be recommended as a substitute for enterocystoplasty.

Acute and Chronic pBOO
Mild pBOO, 35 cmH2O
Severe pBOO, 70 cmH2O

Normal Bladder Resting Pressure
20 cmH2O
Bladder Augmentation with BLSF Grafts in a Porcine pBOO Model

Bladder Defect

Graft Implantation

Cross-section of New Bladder Tissue

Muscle

Epithelium

New Bladder Tissue (3 months)

Exterior

Interior

Post-pBOO

Repair (3 months)

100% Increase in Bladder Capacity over pBOO
330% Increase in Bladder Compliance over pBOO
Urethral Stricture Disease

- A urethral stricture is scarring in or around the urethra that narrows or blocks urine flow.

- Urethral strictures can result from trauma (straddle injury), infection (STD), and chronic inflammation (lichen sclerosus).

- Relatively common disease in males (~400 per 100,000) with increased incidence after 55 years of age.

- 1.5 million outpatient visits per year. Complications including stones, incontinence, infertility, and renal damage.

Surgical Strategies for Urethral Repair

• Endoscopic approaches for urethral stricture repair
  o Dilation or incision (cold knife internal urethrotomy)
  o 100,000-235,000 procedures performed annually in the US*
  o Success rate of dilation is low and typically unsuccessful for >1 cm strictures
  o Repeat internal urethrotomy offers no chance of cure after 3rd treatment or restructre in 3 months.

• Open Urethroplasty
  o Approach based on length and severity of the defect
  o End to end anastomosis (stricture) versus onlay urethroplasty with autologous tissue grafts (stricture and hypospadias)
  o Treatment limited to highly specialized centers, ~2500 procedures in the US annually

Preclinical Trials for Urethral Reconstruction

Rabbit Stricture Model  Urethral Implantation

Cross-section of New Urethral Tissue

Muscle  Epithelium

Stricture  Repair (3 months)

Restoration of 80% Urethral Caliber
Lessons Learned from the Urinary Tract Reconstruction

Incomplete Degradation

Stone Formation/Obstruction

Persistent Fibrosis

Global

Magnified

Normal

Obstructed

Next Generation Prototypes
Enhanced Degradation Kinetics
Anti-fibrotic Drug Delivery Capacity
Esophageal Diseases and Prevalence

Atresia/TEF
1 in 4425 births

Strictures
23% of patients with reflux

Esophageal Cancer
6th leading cause of cancer death

Barrett’s Esophagus
20% of patients with reflux

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Current Treatment Options and Complications

Gastric Pull-up

Colonic Interposition

- Anastomotic leakage (12-29%)
- Strictures (19-53%)
- Dysmotility and dysphagia (5-25%)
- Donor site morbidity (26-55%)
- Death (3-6%)

~700 procedures/year in US

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Performance of BLSF Grafts in Animal Models of Esophageal Repair

Onlay Esophagoplasty

- **BLSF**
- **SIS**

**Rat Models**
- Algarrahi et al., 2015
- Algarrahi et al., 2018a

**Swine Models**
- Algarrahi et al., 2018b
- Gundogdu et al., 2020

Onlay/Tubular Esophagoplasty

**BLSF Repair - 3 months**
Understanding Mechanisms of Neotissue Remodeling and Identifying Scaffold Independent Control Points are Key to Maximizing Functional Performance

Rat Onlay Esophagoplasty

- Scaffold Degradation
- Neotissue Remodeling

Gross (MTS)

RA (MTS)

Cluster 3

- Neuregulin Signaling
- Acute Myeloid Leukemia Signaling
- Glia Signaling
- G Beta Gamma Signaling
- RANK Signaling in Osteoclasts
- IL-3 Signaling
- GDNF Family Ligand-Receptor Interactions
- ErbB Signaling
- NKJ Signaling
- Estrogen-Dependent Breast Cancer Signaling
- GM-CSF Signaling
- Anti-proliferative Role of Somatostatin Receptor 2
- Thrombopoietin Signaling
- Progesterone Signaling
- ERK/MAPK Signaling
- HGF Signaling
- JAK/Stat Signaling
- IL-2 Signaling
- Induction of Apoptosis by HIV
- Oncostatin M Signaling
- Mitotic Roles of Polo-Like Kinase
- Aldosterone Signaling in Epithelial Cells
- UVA-induced MAPK Signaling
- EGF Signaling
- FAS Signaling
- PVR Signaling
- P2X3 Signaling
- AGR2 Signaling
- Ang II Signaling at Neuroneuronal Junction
Transient Stenting is Necessary to Support Remodeling of Tubular BLSF Grafts

Transient Stenting for 2 months reduced the rate of esophageal strictures from 100% to 60%
Not all implant sites are created equal and regeneration is often heterogeneous.

- **Graft Periphery**
- **Graft Center**
- **Rat Onlay Ileoplasty 2 months**
- **Scar Tissue Formation**
- **Neotissue**

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Pediatric and Adult Tracheal Diseases

Spectrum of pathological phenotypes of the tracheal cartilages

<table>
<thead>
<tr>
<th>Normal</th>
<th>Tracheal Agenesis</th>
<th>Tracheal Malacia</th>
<th>Tracheal Stenosis</th>
<th>Tracheal Cartilaginous Sleeve</th>
<th>Tracheo-oesophageal Fistula</th>
</tr>
</thead>
</table>

- Normal: Hy, Th, Cr, Al, Ca, Br, St
- Tracheal Agenesis: Oe
- Tracheal Malacia: Th, Cr, Al, Ca, Br, St
- Tracheal Stenosis: Th, Cr, Al, Ca, Br, St
- Tracheal Cartilaginous Sleeve: Th, Cr, Al, Ca, Br, St
- Tracheo-oesophageal Fistula: Th, Cr, Al, Ca, Br, St

Trachea - Tissue Composition

- Goblet cell
- Pseudostratified ciliated columnar epithelium
- Lamina propria (connective tissue)
- Submucosa
- Seromucous gland in submucosa
- Hyaline cartilage

Photomicrograph of the tracheal wall (320x)
Rat Onlay Tracheoplasty with BLSF Grafts: Success and Challenges

Next Generation Prototypes
Enhanced Degradation Kinetics
Improved Cartilage Formation

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Conclusions

• BLSF grafts are capable of supporting the formation of innervated, vascularized tissues across multiple preclinical models of hollow organ reconstruction.

• Validation of silk fibroin grafts in preclinical models mimicking patient pathology is necessary to optimize functional performance.

• Enhanced control of in vivo scaffold degradation, improved anti-fibrotic properties, and increased understanding of signaling mechanisms responsible for neotissue formation is crucial in developing translational matrix prototypes.
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