ESOPHAGEAL ATRESIA:
WHAT WE KNOW AND WHAT
IS ON THE HORIZON
BUILDING BRIGHTER FUTURES
WESTBROOK, CT

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• Innovation studies of tissue engineered replacement designed in conjunction with Biostage

• Study supported by NIH R44HD095784 and Biostage funds
THE PROBLEM: BABIES BORN WITH A GAP IN THEIR ESOPHAGUS

- 3.9 million births per year in US
- 100 children per year have a very large gap that is difficult to treat

The esophagus is a tubular structure, it appears simple in design but is complicated in function.

Congenital or acquired esophageal disorders can be devastating for children.

Current surgical strategies are associated with 20-40% incidence of morbidity and prolonged hospital stay.
Types of Esophageal Atresia

C: 87%
A: 8%
E: 4%
D: 1%
B: 1%
Diagnosis

Polyhydramnios

Microgastria

GA = 20 wks 2 days
Know the Disease

• Patients with EA are 364-fold more likely to develop eosinophilic esophagitis compared to the general population [1].

• ESPGHAN-NASPGHAN guideline recommends routine endoscopy in adults born with EA [2].
  o 4 EA patients developed carcinoma of the gastrointestinal tract

• **Lifelong screening of the upper gastrointestinal tract in EA patients.**


# Associated Anomalies

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Phenotype</th>
<th>Genes Associated</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>VACTERL</td>
<td>Multiple anomalies: vertebral, renal, cardiac, radial, anorectal, EA/TEF</td>
<td></td>
<td>[8, 16]</td>
</tr>
<tr>
<td>Anophthalmia-esophageal-genital syndrome</td>
<td>Esophageal atresia and urogenital anomalies</td>
<td>SOX2</td>
<td>[3]</td>
</tr>
<tr>
<td>CHARGE syndrome</td>
<td>Genital, ear, cardiac, and eye anomalies as well as EA/TEF</td>
<td>CHD7</td>
<td>[3]</td>
</tr>
<tr>
<td>Feingold syndrome</td>
<td>Cardiac defects, learning disabilities, and EA/TEF</td>
<td>MYCN</td>
<td>[3]</td>
</tr>
<tr>
<td>Fanconi anemia syndrome</td>
<td>Anomalies affecting bones, renal system, central nervous system, hearing, cardiac defects, EA/TEF, and other developmental delays</td>
<td>20 genes</td>
<td>[3]</td>
</tr>
<tr>
<td>Chromosomal anomalies</td>
<td>Variable phenotype depending on chromosome involved</td>
<td>Trisomy 21, Trisomy 18, Trisomy 13, Trisomy 10</td>
<td>[16, 18]</td>
</tr>
</tbody>
</table>
Surgery for Esophageal Atresia/TEF
Repair of TEF
What happens when you cannot get the ends together?
Options for LGEA

- Delayed repair
- Interposition graft - stomach, jejunum, colon
- Esophageal elongation procedure
- Foker Technique
- Innovation
  - Magnet
  - Tissue engineered esophagus
<table>
<thead>
<tr>
<th>Study</th>
<th>Procedure(s)</th>
<th>Primary Complication(s) (Rates)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stadil et al. 2018</td>
<td>Delayed primary closure (DPR) vs esophageal replacement (ER) (gastric pull-up, gastric tube, colonic interposition, or jejunal interposition)</td>
<td>Anastomotic leak: DPR 32.4%, ER 38.2% (p&gt;0.05) Dilations: DPR 62.2%, ER 73.5% (p&gt;0.05) GERD: DPR 67.6%, ER 38.2% (p&lt;0.05) Reoperation: DPR 10.8%, ER 11.8% (p&gt;0.05) Postoperative mortality: DPR 0%, ER 2.9% (p&gt;0.05)</td>
<td>[51]</td>
</tr>
<tr>
<td>Leibovitch et al. 2018</td>
<td>Primary repair, delayed primary closure, esophageal replacement with gastric pull-up</td>
<td>Anastomotic stricture: 74% Dilations: 65% Food bolus impactions: 78% GERD: 91%</td>
<td>[105]</td>
</tr>
<tr>
<td>Liu et al. 2017</td>
<td>Colonic interposition (CI), Gastric pull-up (GP), Jejunal Interposition (JI), Gastric tube Reconstruction (GTR)</td>
<td>Anastomotic stricture: CI 15.2%, GP 26%, JI 20%, GTR 48.1% GERD: CI 13.7%, GP 16.5%, JI 6.7%, GTR 48.1% Anastomotic Leak: CI 19.7%, GP 22.8%, JI 37.8%, GTR 25.9% Respiratory Problems: CI 14.3%, GP 11%, JI 22.2%, GTR 29.6%</td>
<td>[50]</td>
</tr>
<tr>
<td>Li et al. 2017</td>
<td>Delayed primary closure (DPR), Jejunal interposition (JI)</td>
<td>Anastomotic leak: 4.5% Respiratory failure: 21.2%</td>
<td>[106]</td>
</tr>
</tbody>
</table>
# Mechanical Stretching

<table>
<thead>
<tr>
<th>Study</th>
<th>Procedure</th>
<th>Outcomes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bairdain et al. 2013</td>
<td>Mechanical tension system (hydrostatic) for traction in LGEA Multi-institutional</td>
<td>7 patients; Median time to repair 15 days; 3 patients mechanical entrapment; All strictures requiring dilation</td>
<td>[104]</td>
</tr>
<tr>
<td>Nasr et al. 2013</td>
<td>Delayed repair vs primary Foker; 71 Foker patients; 451 patients delayed repair Review and Meta-analysis</td>
<td>Foker: lower risk of complications and significantly shorter time to anastomosis</td>
<td>[60]</td>
</tr>
<tr>
<td>Sroka et al. 2013</td>
<td>Kimura vs Foker technique Group A gap 6 cm (n=6): traction on both pouches; Group B gap 9.5 cm (n=6): Kimura upper pouch; traction on lower pouch; Group C gap 6.5 cm (n=3): close spit fistula and traction on both</td>
<td>Group A: traction time 3 weeks; 50% stricture; 2.1 thoracotomies; Group B: traction time 48-143 weeks (infection); 83% leak; 3.6 thoracotomies; 33% stricture; Group C: 100% leak; successful anastomosis in only 1</td>
<td>[59]</td>
</tr>
<tr>
<td>Bairdain et al. 2015</td>
<td>Retrospective review 27 primary Foker gap 4.5 cm; 25 secondary Foker (had failed repair first) gap 5.0 cm</td>
<td>Primary: 63% full oral nutrition; 0% mortality; 70 ICU days; Secondary: 9% full oral nutrition; 8% mortality; 110 ICU days</td>
<td>[56]</td>
</tr>
</tbody>
</table>
Innovative Solutions

Oral catheter
1. Magnet
2. Suction port
Gastric catheter
3. Magnet
4. Balloon inflation port
5. Feeding & medication port
6. Bolster (outer anchor)
Histology of Normal Esophagus

This section shows normal esophageal squamous mucosa at the upper, with underlying submucosa containing mucus glands and a duct surrounded by lymphoid tissue. The muscularis is at the lower.
*Compilation of POC and 3 GLP Porcine Studies-ADULT MODEL*
<table>
<thead>
<tr>
<th>Amount of Fat</th>
<th>P1 Cell Yield</th>
<th>P2 Cell Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.962g</td>
<td>47 M</td>
<td>31.68 M</td>
</tr>
<tr>
<td>10.932g</td>
<td>30.72M</td>
<td>58.59M</td>
</tr>
<tr>
<td>9.861g</td>
<td>12.9 M</td>
<td>20.07 M</td>
</tr>
<tr>
<td>13.239g</td>
<td>21.2 M</td>
<td>47.52 M</td>
</tr>
</tbody>
</table>

Scaffold Incubated for 6 days in Culture
In Vitro Analysis of Seeded Scaffold After 6 Days
Results Day 21: EGD and stent removal
Harvest: Gross Histology
Gross histology at 30 days

Unseeded Scaffold
Masson’s Trichrome staining – Day 90

18P0634  CEI implanted

18P0636  Procedural Control

66-013  Non-Seeded CEI
Fate Study Utilizing GFP Tagged Porcine AD-MSCs

- Box 1 demonstrates lack of GFP staining in the representative luminal area of the neotissue.
- Box 2 showing scattered GFP staining with a prominent perivascular distribution. 2a.
- Box 3 showing higher density of GFP positive cells with prominent perivascular distribution and GFP+ cells lining vascular structures.

Source: William Fodor, PhD - Biostage
Accepted Abstract ISSCR 2019
Compassionate Use

"On May 4, 2017, the Cellspan Esophageal Implant was surgically implanted into a 75-year old male with a life-threatening cancerous mass in his chest. The surgery was required to address the tumor's encroachment on the patient's lung, heart, and esophagus. The portion of the esophagus affected by the cancer was removed and the Cellspan Esophageal Implant was utilized to reconstruct the esophagus. The patient survived more than three months and succumbed to issues unrelated to the implant. Autopsy showed that the implant was in place and regenerating. Biostage believes that the Cellspan Esophageal Implant has performed as designed."
Is this translatable?

Presented at the CT Chapter of the American College of Surgeons
October 2019
1. Determine the feasibility of isolating and expanding AD-MSCS from normal and EA patients
2. Determine the viability and metabolic activity of expanded cells seeded onto manufactured scaffolds
3. Measure key differences between gene expression and cytokine production of the cells
A NOVEL AUTOLOGOUS TISSUE ENGINEERED TUBULAR ORGAN REPLACEMENT

2-D Cell Culture Expansion

Automatic Cell Seeding and closed System 3-D BioRx: Final Product Incubation

- Metabolic Profile & Viability
- Cell Quantity-DNA Assay
- MOA Characteristics
  - Secretome Profile

Bioreactor ‘In Process’ Monitoring

Cellspan Esophageal Implant

SEM (2000X)

CIG Diagram of Critical CEI Spec’s
## Characterization of hpAD-MSCs

<table>
<thead>
<tr>
<th>Age</th>
<th>Gender</th>
<th>Condition</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt 1</td>
<td>16</td>
<td>Male</td>
<td>Normal</td>
</tr>
<tr>
<td>Pt 2</td>
<td>19</td>
<td>Female</td>
<td>Normal</td>
</tr>
<tr>
<td>Pt 3</td>
<td>14</td>
<td>Female</td>
<td>Normal</td>
</tr>
<tr>
<td>Pt 4</td>
<td>2 Days</td>
<td>Female</td>
<td>TEF</td>
</tr>
<tr>
<td>Pt 5</td>
<td>6 Days</td>
<td>Female</td>
<td>Normal</td>
</tr>
<tr>
<td>Pt 6</td>
<td>7 Days</td>
<td>Male</td>
<td>TEF</td>
</tr>
<tr>
<td>Pt 7</td>
<td>2 Days</td>
<td>Male</td>
<td>LGEA</td>
</tr>
<tr>
<td>Pt 8</td>
<td>2 Days</td>
<td>Female</td>
<td>TEF</td>
</tr>
</tbody>
</table>

![Relative Gene Expression Chart](chart.png)
Scaffold Metabolism & Cell Viability

Green = Live  Red = Dead

![Graphs showing changes in glucose and lactate levels over culture days for different groups.](image)
Cytokine Production & Gene Expression of hpAD-MSCs on Scaffolds
Conclusions

• Esophageal Atresia is a complex problem - it is important to know it is a lifelong condition

• Long gap esophageal atresia affects 100 neonates annually

• Current surgical strategies are fraught with significant morbidity

• Innovative techniques are being explored to improve outcomes
Conclusions

- Pilot studies demonstrate feasibility of implanting a cell seeded synthetic scaffold to bridge a long esophageal gap in a pediatric animal model.

- Scaffold is extruded by 21 days postoperatively which is important in a pediatric model.

- Initial regeneration appears to be fibrovascular neotissue with evidence of vessel sprouting from the aorta.

- Epithelialization occurs over time.
An Important Step Forward

- Cells do not appear to vary greatly in phenotype between EA patients and normal patients
- The scaffold supports viability and expansion of cells
- Normal Patients appeared to be less metabolically active as indicated by Glucose and Lactate Levels
- **Important Note:** Cell lines were derived from less than 500mg of tissue obtained at the time of surgical intervention
- This combination device is currently being reviewed by the FDA for clinical trials in the US
Next Steps

- FDA Application in process
- Phase II completion of NIH grant demonstrating feasibility
- Potential clinical trials in 2021
CT Children’s & Biostage Team

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