Importance
The study demonstrates use of a novel intervention for severe tracheobronchomalacia (TBM).

Objective
To test a novel, 3-dimensionally (3D) printed, bioresorbable airway splint for efficacy in extending survival in a porcine model of severe, life-threatening TBM.

Design and Participants
A randomized, prospective animal trial was used to evaluate an external airway splint as treatment of severe, life-threatening TBM in a multi-institutional, multidisciplinary collaboration between a biomedical engineering department and an academic animal surgery center. Six 2-month-old Yorkshire pigs underwent tracheal cartilage division and inner tracheal lumen dissociation and were randomly assigned to splint treatment (n = 3) or control groups (n = 3). Two additional pigs had the splint placed over their normal trachea.

Interventions
A 3D-printed, bioresorbable airway splint was assessed in a porcine animal model of life-threatening TBM. The open-cylindrical, bellow-shaped, porous polycaprolactone splint was placed externally and designed to suspend the underlying collapsed airway. Two additional animals were splinted without model creation.

Main Outcomes and Measures
The observer-based Westley Clinical Croup Scale was used to assess the clinical condition of animals postoperatively. Animal survival time was noted.

Results
Complete or nearly complete tracheal lumen collapse was observed in each animal, with resolution of symptoms in all of the experimental animals after splint placement. Using our severe TBM animal model, survival was significantly longer in the experimental group receiving the airway splint after model creation than in the control group (P = .0495).

Conclusions and Relevance
A multidisciplinary effort producing a computer-aided designed, computer-aided manufactured bioresorbable tracheobronchial splint was tested in a porcine model of severe TBM and was found to extend survival time. Mortality in the splinted group was ascribed to the TBM model based on the lack of respiratory distress in splinted pigs, long-term survival in animals implanted with the splint without TBM, and necropsy findings.
Novel Airway Splint for Porcine Tracheomalacia

Original Investigation Research

A collaborative effort between departments of pediatric otolaryngology-head and neck surgery and biomedical engineering produced a novel tracheobronchial splint designed to treat airway malacia at the tracheal and/or bronchial level. The splint had an open cylindrical bellow design with periodically spaced pores to allow suturing of the trachea within the splint. The splint was automatically generated using a custom-written MATLAB program that allowed specification of splint diameter, length, opening angle, suture pore spacing, and bellow wave period, height, and shape. Finite element analysis of compression, 3-point bending, and internal growth was performed on initial designs (Figure 1A-C). The design was generated as image data and directly converted to a surface representation in .STL format (Figure 1D and E) as input data for manufacturing. This .STL file was also imported to a 3-dimensional (3D) model of the subject’s trachea and/or bronchus to allow sizing for a customized fit. The splint was manufactured from PCL with 4% hydroxyapatite using laser sintering (Formiga P 100 system; EOS e-Manufacturing Solutions), a 3D printing technology, that has been adapted to use PCL to build complex 3D structures (Figure 1F). Approximately 200 splints may be built in 4 hours using this manufacturing technology. Prior to surgical implantation, all scaffolds were ethylene-oxide sterilized and allowed to outgas.

Surgical Model of Severe Tracheomalacia

Institutional Animal Care and Use Committee (IACUC) approval was obtained at the University of Illinois. The Yorkshire pig was used as a preclinical animal model because the porcine trachea has biomechanical and anatomic properties similar to those of the growing human trachea. Based on extending the length of survival with intervention, it was determined through statistical power analysis that the minimum number of pigs required was 6 for an α value of 0.8. Two-month-old Yorkshire pigs (n = 6) were randomly assigned to treatment (n = 3) or control groups (n = 3). Two additional pigs had the splint placed over their normal trachea. Polycaprolactone scaffolds were customized to fit porcine trachea from animals weighing approximately 20 kg.

Each pig underwent surgical creation of the model for tracheomalacia illustrated in Figure 2. Intravenous ketamine, telatamine/zolazepam, xylazine, and atropine followed by inhaled isoflurane, 3% to 5%, were used for anesthetic. The volume of the anesthetic mixture was 0.1 mL/kg or 2 mL for 20 kg. Body temperature, heart rate, and breathing rate as well as pulse rate were monitored to assure the appropriate depth of anesthesia.

The cervical skin was prepared and draped in sterile fashion. An anterior cervical approach via a vertical, midline skin incision over the larynx and trachea was performed. The sternothyroid and sternohyoid musculature, thyroid, and cervical thymus were dissected and retracted laterally, providing wide exposure to the trachea. A malacic segment was produced by extraluminal resection of tracheal ring 3 followed by subperichondrial dissection of the next 4 consecutive tracheal rings. To ensure nearly complete or complete dynamic collapse, lateral dissection of the internal tracheal mucosal was carried posterolaterally and inferiorly allowing nearly complete dissociation from external tracheal layers resulting in complete flaccidity of the airway segment. Care was taken to limit endoluminal tears, though if encountered, a single, interrupted 4-0 Vicryl suture (Ethicon Inc) was used for repair. With the internal mucosa protected, the overlying tracheal rings were sharply divided, creating 4 inferiorly based, distinct, narrow strips. Visually, severe collapse was evident intraoperatively after model creation noted by external observation. The surgical sites of the 3 control animals were then closed.

In the experimental group, the tracheomalacia model was created as in the control animals, and then each animal had a 14-mm internal diameter PCL splint placed external to the trachea. The internal mucosa, or surgically created malacic segment, was suspended to the external PCL airway splint using 4-0 Vicryl or Prolene suture (Ethicon Inc) passed through pre-

Methods

Splint Design

A collaborative effort between departments of pediatric otorhinolaryngology and head and neck surgery and biomedical engineering produced a novel tracheobronchial splint designed to treat airway malacia at the tracheal and/or bronchial level. The splint had an open cylindrical bellow design with periodically spaced pores to allow suturing of the trachea within the splint. The splint was automatically generated using a custom-written MATLAB program that allowed specification of splint diameter, length, opening angle, suture pore spacing, and bellow wave period, height, and shape. Finite element analysis of compression, 3-point bending, and internal growth was performed on initial designs (Figure 1A-C). The design was generated as image data and directly converted to a surface representation in .STL format (Figure 1D and E) as input data for manufacturing. This .STL file was also imported to a 3-dimensional (3D) model of the subject’s trachea and/or bronchus to allow sizing for a customized fit. The splint was manufactured from PCL with 4% hydroxyapatite using laser sintering (Formiga P 100 system; EOS e-Manufacturing Solutions), a 3D printing technology, that has been adapted to use PCL to build complex 3D structures (Figure 1F). Approximately 200 splints may be built in 4 hours using this manufacturing technology. Prior to surgical implantation, all scaffolds were ethylene-oxide sterilized and allowed to outgas.

Surgical Model of Severe Tracheomalacia

Institutional Animal Care and Use Committee (IACUC) approval was obtained at the University of Illinois. The Yorkshire pig was used as a preclinical animal model because the porcine trachea has biomechanical and anatomic properties similar to those of the growing human trachea. Based on extending the length of survival with intervention, it was determined through statistical power analysis that the minimum number of pigs required was 6 for an α value of 0.8. Two-month-old Yorkshire pigs (n = 6) were randomly assigned to treatment (n = 3) or control groups (n = 3). Two additional pigs had the splint placed over their normal trachea. Polycaprolactone scaffolds were customized to fit porcine trachea from animals weighing approximately 20 kg.

Each pig underwent surgical creation of the model for tracheomalacia illustrated in Figure 2. Intravenous ketamine, telatamine/zolazepam, xylazine, and atropine followed by inhaled isoflurane, 3% to 5%, were used for anesthetic. The volume of the anesthetic mixture was 0.1 mL/kg or 2 mL for 20 kg. Body temperature, heart rate, and breathing rate as well as pulse rate were monitored to assure the appropriate depth of anesthesia.

The cervical skin was prepared and draped in sterile fashion. An anterior cervical approach via a vertical, midline skin incision over the larynx and trachea was performed. The sternothyroid and sternohyoid musculature, thyroid, and cervical thymus were dissected and retracted laterally, providing wide exposure to the trachea. A malacic segment was produced by extraluminal resection of tracheal ring 3 followed by subperichondrial dissection of the next 4 consecutive tracheal rings. To ensure nearly complete or complete dynamic collapse, lateral dissection of the internal tracheal mucosal was carried posterolaterally and inferiorly allowing nearly complete dissociation from external tracheal layers resulting in complete flaccidity of the airway segment. Care was taken to limit endoluminal tears, though if encountered, a single, interrupted 4-0 Vicryl suture (Ethicon Inc) was used for repair. With the internal mucosa protected, the overlying tracheal rings were sharply divided, creating 4 inferiorly based, distinct, narrow strips. Visually, severe collapse was evident intraoperatively after model creation noted by external observation. The surgical sites of the 3 control animals were then closed.

In the experimental group, the tracheomalacia model was created as in the control animals, and then each animal had a 14-mm internal diameter PCL splint placed external to the trachea. The internal mucosa, or surgically created malacic segment, was suspended to the external PCL airway splint using 4-0 Vicryl or Prolene suture (Ethicon Inc) passed through pre-
fabricated and designed needle holes in the splint. Four sutures were placed at the superior, inferior, and lateral aspects of the airway, with additional sutures placed as necessary. The malacic trachea was then suspended as the sutures were sequentially tied. The surgical sites of the 3 experimental animals were then closed. Antibiotic therapy, ceftiofur, 5.0 mg/kg, was administered intraperitoneally.

To differentiate the effects of the splint from the effects of surgically created tracheomalacia, a third group of animals underwent similar exposure of the trachea. The splint was then directly sutured to the intact trachea.

**Postoperative Evaluation**

Postoperatively, temperature, appetite, behavior, and tenderness at the incision and implant sites were monitored. Furthermore, the validated Westley Group Scale was used for daily clinical assessments of the animals (Table 1). Scores for the control group were not included because survival duration did not extend beyond the first postoperative day. Overnight mortality was assigned a time of death of 23:59pm. To determine whether the differences seen between the 2 groups were statistically significant, a Wilcoxon rank sum test was performed. Statistical significance was defined as \( P < .05 \).

**Results**

At examination, the piglet tracheal rings were found to overlap in a spiral fashion posteriorly preventing collapse. The present model of surgically created tracheomalacia was created based on experiments with cadaveric pig tracheas.

Pig mean weight was 21 kg (range, 16-25 kg). No animals exhibited respiratory symptoms prior to surgery (Westley Score, 0). Complete or nearly complete inner lumen collapse was confirmed in all 6 pigs with surgically created tracheomalacia. The tracheomalacia was accompanied by severe audible inspiratory stridor in all cases. One pig was excluded secondary to an intraoperative emesis and aspiration event on induction prior to surgical model creation (this pig would have been in the control arm). There were no other intraoperative deaths or complications.

All 3 pigs with untreated surgically created tracheomalacia displayed severe stridor, cyanosis, and retractions postoperatively. The decision to kill was made when level of consciousness changes were displayed. Daily postoperative clinical Westley Scale scores are reported in Figure 3. The animal with the longest survival with surgically created tracheomalacia,
into postoperative day 7, received the splint and had a West-ley clinical score rising from 3, peaking at 11, and stabilizing in the moderate range. The 2 additional animals receiving the splint for surgically created tracheomalacia died between post-operative days 3 and 4 with minimal clinical signs of respiratory distress. The 2 pigs without surgically created tracheomalacia survived without symptoms over 6 months.

Postmortem examination of the splinted pigs with surgically created tracheomalacia demonstrated peritracheal infection.

Overall duration of survival is detailed for each animal in Table 2. Survival in the control group with untreated surgically created tracheomalacia had a range of 1 hour to 20 hours, 39 minutes. Survival in the splinted group with surgically created tracheomalacia was significantly longer ($P = .0495$), with a range of 84 hours, 14 minutes, to 167 hours, 9 minutes. No mortality was observed in splinted animals without surgically created tracheomalacia.

**Discussion**

Our report examines the efficacy of a computer-aided designed and manufactured, 3D-printed, bioresorbable external tracheal splint designed to treat pediatric tracheomalacia. The PCL laser-sintered splint demonstrated a statistically significant survival benefit in a porcine model of severe tracheomalacia.

Current options available to address severe tracheomalacia, recalcitrant to positive-pressure ventilation and tracheostomy, aim to suspend the flaccid airway segment, restore rigidity, or in the case of segmental resection, remove the weakened area. These interventions have high rates of complications, morbidity, and mortality and have yet to be subjected to randomized trials. More recent reports support the use of aortopexy, with high rates of resolving acute life-threatening events in experienced hands; however, treatment failures and complications such as phrenic and recurrent laryngeal nerve injury have been reported. Complications of internal tracheal stents include stent migration, infection, erosion of nearby structures, need for revision procedures to modify or remove the stent, and secondary obstruction from reactive granulation. In 2005, a US Food and Drug Administration notification cautioned against the use of metal tracheal stents for these reasons.

Rainer et al in 1968, and later Filler et al, described the use of external tracheal Marlex mesh (Phillips Petroleum Company) and Dacron-reinforced Silastic for tracheal splinting in humans (Dupont Teijin Films US [Dacron] and Dow Corning Corporation [Silastic]). Further investigation of external splinting was performed in animal models. Nalwa et al demonstrated complete tracheal mucosal collapse after resecting 3 interrupted segments from 6 consecutive tracheal rings with the subsequent application of a PLPG splint externally (poly-1-lactic acid-polyglycolic acid). Shaha et al resected 6 to 7 consecutive tracheal rings and applied external polytetrafluoro-ethylene grafts. These animals were reportedly free from signs of respiratory distress postoperatively, though no control animals were included in these studies; thus, it would be difficult to judge what postoperative respiratory distress would...
have been observed. Robey et al\(^3\) described an external bioresorbable tracheal splint composed of 85:15 poly(lactide-co-glycolide) (PLGA) applied to a porcine model but with premature splint degradation and loss of airway support. Our initial attempts using models similar to those used by Nalwae et al\(^5\) and Shaha et al\(^13\) did not maintain signs of respiratory distress postoperatively and would not likely have been able to delineate significant clinical benefit of intervention. An essential factor in producing severe tracheomalacia symptoms that were not transient was nearly complete anterior and lateral dissociation of inner tracheal mucosa. Otherwise, control animals had an unremarkable postoperative course.

The severity of our tracheomalacia model allowed for the assessment of airway splint efficacy in survival. Infection secondary to intraluminal needlehole communications was likely responsible for the deaths of the splinted pigs. It is notable that 2 of the 3 experimental animals had Westley scores of 1 and 2 (ie, excellent clinical appearance) prior to death. A modification to our model, produced subsequent to the model discussed in the current report, maintained an anterior island of full-thickness trachea, including cartilage, allowing for avoidance of mucosal needle holes, though still with extensive posterolateral mucosal dissection and nearly complete collapse. This animal had cardiopulmonary arrest postoperatively and was returned to the operating room for emergency splint placement. Approximately 8 weeks postoperatively, there was no sign of infection, and the animal maintained excellent clinical appearance. Without surgically created tracheomalacia, animals with splints placed around their tracheas were asymptomatic with long-term survival.

Several aspects of our splint engineering have been meticulously examined and tailored, with the aid of computational design and 3D fabrication, to treat pediatric tracheobronchomalacia. The splint is designed to provide sufficient rigidity to maintain airway patency while allowing internal expansion necessary for tracheal growth. Our laser sintering process is able to rapidly fabricate splints with a defined external shape, internal pore size, and architecture. The bioresorbable nature of the splint is intended to allow for growth of the native trachea while avoiding additional surgical and anesthetic exposures. The biomaterial used to construct the splints, PCL, is specifically chosen for its proven ability to maintain structural integrity for greater than 24 months in human clinical trials.\(^14\) This duration ideally matches the time generally required for growth and development of the trachea sufficient for resolution of symptoms in tracheomalacia\(^15\). Furthermore, PCL material

### Table 1. Animal Daily Clinical Scoring System Based on the Westley Clinical Croup Scale

<table>
<thead>
<tr>
<th>Signs, Symptoms, and Indications</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcostal retractions</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Mild</td>
<td>1</td>
</tr>
<tr>
<td>Moderate</td>
<td>2</td>
</tr>
<tr>
<td>Severe</td>
<td>3</td>
</tr>
<tr>
<td>Stridor</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>With agitation</td>
<td>1</td>
</tr>
<tr>
<td>At rest</td>
<td>2</td>
</tr>
<tr>
<td>Cyanosis</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>With agitation</td>
<td>4</td>
</tr>
<tr>
<td>At rest</td>
<td>5</td>
</tr>
<tr>
<td>Level of consciousness</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td>Disoriented</td>
<td>5</td>
</tr>
<tr>
<td>Air entry</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td>Decreased</td>
<td>1</td>
</tr>
<tr>
<td>Markedly decreased</td>
<td>2</td>
</tr>
</tbody>
</table>

### Table 2. Duration of Survival in Animals With a Model of Severe Tracheomalacia With and Without an Interventional Airway Splint

<table>
<thead>
<tr>
<th>Subject Pig</th>
<th>Weight, kg</th>
<th>Survival Time, h:min:s*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>24</td>
</tr>
<tr>
<td>Splinted</td>
<td>4</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>20</td>
</tr>
</tbody>
</table>

*P = .0495 for the difference in survival time between splinted and control animals.

#### Figure 3. Postoperative Cumulative Westley Clinical Croup Scale Score in Splinted Animals

Each line represents an individual experimental animal, each geometric figure a measurement point. X indicates death at the time of measurement or during previous time interval. The X at postoperative day 1 represents all control animals (n = 3), indicating that no control animal survived beyond 24 hours. The rightward pointing arrow across the top of the graph represents long-term survival in the 2 animals with implanted splints placed without tracheomalacia.
Novel Airway Splint for Porcine Tracheomalacia

Original Investigation Research

Statistical analysis: Hollister, Green.

The article was accepted for podium presentation and interpretation of the data; and preparation, review, study; collection, management, analysis, and Health had no role in the design and conduct of the study. Role of the Sponsor: 2UL1TR000433-06 (Drs Hollister and Green).


Conflicts of Interest: Zopf, Flanagan, Wheeler, Green. Critical revision of the manuscript for important intellectual content: Zopf, Flanagan, Wheeler, Hollister, Green. Statistical analysis: Zopf, Wheeler. Obtained funding: Green. Administrative, technical, or material support: Zopf, Flanagan, Wheeler, Green. Study supervision: Zopf, Wheeler, Hollister, Green. Conflict of Interest Disclosures: Drs Hollister and Green have filed a patent application and have a patent pending on the device described in this article (patent pending No. 13/715,715; June 20, 2013), titled “Porous Bidirectional Bellowed Tracheal Reconstruction Device.” No other conflicts have been reported.

ARTICLE INFORMATION
Submitted for Publication: April 10, 2013; final revision received September 10, 2013; accepted September 18, 2013. Published Online: November 14, 2013. doi:10.1001/jamaoto.2013.5644.Autor Contributions: Drs Zopf and Green had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Zopf, Wheeler, Hollister, Green. Acquisition of data: Zopf, Wheeler, Hollister, Green. Analysis and interpretation of data: Zopf, Flanagan, Wheeler, Hollister, Green. Drafting of the manuscript: Zopf, Wheeler, Green. Critical revision of the manuscript for important intellectual content: Zopf, Flanagan, Wheeler, Hollister, Green. Statistical analysis: Zopf, Wheeler. Obtained funding: Green. Administrative, technical, or material support: Zopf, Flanagan, Wheeler, Green. Study supervision: Zopf, Wheeler, Hollister, Green. Conflict of Interest Disclosures: Drs Hollister and Green have filed a patent application and have a patent pending on the device described in this article (patent pending No. 13/715,715; June 20, 2013), titled “Porous Bidirectional Bellowed Tracheal Reconstruction Device.” No other conflicts have been reported.

Funding/Support: This work was supported by National Institutes of Health grant ZUL1TR000433-06 (Drs Hollister and Green).

Role of the Sponsor: The National Institutes of Health had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data, and preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Previous Presentation: The manuscript for this article was accepted for podium presentation and awarded the William P. Potsic First Place Research Award at the American Society of Pediatric Otolaryngology 2013 Spring meeting. April 26-28, 2013; Arlington, Virginia.

Additional Contributions: We would like to acknowledge Hyungjin Kim, ScD, Department of Biostatistics, University of Michigan, for her statistical assistance; Chanaka Rabel, PhD, Department of Animal Sciences, University of Illinois, Urbana-Champaign, Aaron Maki, PhD, Department of Bioengineering, University of Illinois, Urbana-Champaign, James Cooper, University of Illinois, Urbana-Champaign, Anna Ercolin, DVM, Department of Animal Sciences, University of Illinois, Urbana-Champaign, and Kelly Roballo, Department of Animal Sciences, University of Illinois, Urbana-Champaign, for collection of the post-surgical respiratory data and animal monitoring; and especially Jonathan F. Mosley, Department of Animal Sciences, University of Illinois, Urbana-Champaign, along with his staff at the Physiology Research Laboratory/Imported Swine Research Laboratory, for the animal management, surgery assistance, and excellent animal care.

REFERENCES