Use of Internal Bioabsorbable PLGA “Finger-Type” Stents in a Rabbit Tracheal Reconstruction Model

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Objectives: To design and develop a biodegradable tracheal stent that can be used internally to stabilize and support surgically reconstructed airways.

Design: In vitro mechanical and degradative properties of 80:20 poly(D,L-lactide-co-glycolide) (PLGA) “finger-like” stents were determined. The stents were then tested in vivo in rabbits that underwent anterior patch tracheoplasties with fascia lata grafts. Comparisons were made between a control group and an internal stent group for stridor development, overall group mortality, reconstructed airway lumen size, and histological findings.

Subjects: Twenty-five New Zealand white rabbits.

Results: The average dry modulus for the internal stents was 6800 kPa. All of the internal stents cracked by 4 weeks in buffer solution. Significant mass loss was not noted in vitro until after 5 weeks in buffer solution. By 14 weeks, the stents were nearly 100% degraded. The attrition rate for the control group was 23% compared with 17% for the experimental group. The stridor rate for the control group was also higher at 38% compared with 17% for the stented group. The stented rabbits had a significantly smaller average stenosis (23%) across the entire reconstruction site than the control group (34%) (P<.05).

Conclusion: Biodegradable PLGA stents degrade in a predictable fashion and have a statistically significant effect in augmenting anterior patch tracheoplasties with fascia lata grafts in rabbits.

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RACHICAL STENTS have served as both primary treatment modalities for obstructive airway conditions and stabilizers to other surgical reconstructive procedures. The purposes of stenting in the airway are to counteract scar contracture and to promote a scaffold for epithelium to cover the reconstructed lumen of the airway. When used in conjunction with a tracheal reconstructive procedure, stenting also holds the reconstructed segment in place and prevents mechanical disruption secondary to movement of the trachea during breathing and swallowing. The ideal stent should be simple to insert, fix, and remove; be biocompatible; not obstruct the airway; allow clearance of secretions; and accommodate to varying tracheal dimensions and shapes.

A wide variety of airway stents has been reported in the literature, but no consensus has been reached regarding the best stenting option. Montgomery’s silicone T-tube stent was the first internal tracheal stent to be widely accepted. Despite the widespread use of this stent and other silicone stents, complications have been seen with their long-term use, including impairment of mucociliary clearance, further narrowing of the airway by the wall of the stent, migration of the stent, breakage of the stent, and the inability of the stent to grow with the trachea. It is also difficult to intubate a patient who has a silicone stent in place.

Wallace et al introduced an expandable stainless steel stent, based on Gianuturo’s endovascular zigzag stent, that can be placed endoscopically in the tracheobronchial tree. Numerous authors have subsequently reported using this stent clinically, most often in the lower airway. However, metallic expandable stents are not amenable to temporary intraluminal placement since fixation of their stent hooks to the mucosal wall makes their removal difficult. They are possible to remove endoscopically if broken and unraveled with the aid of a bronchoscope, but this comes with a high risk of tracheobronchial wall perforation. Other complications reported include tra-
METHODS AND MATERIALS

The internal finger stents were fabricated using a patented process at the Bionx Implants facilities in Tampere, Finland. The internal finger stents were made of the bioabsorbable copolymer, poly(D,L-lactide-co-glycolide) (PLGA) (80:20). Homogeneous 80:20 PLGA internal finger stents were produced with a fiber diameter of 0.4 mm, an outer diameter of 0.6 mm, and a length of approximately 2.0 cm (Figure 1). The fingers were closed in a 270° fashion, allowing for a gap to be maintained posteriorly, thus, giving an effective internal diameter of approximately 3.5 mm. Each of the stents was sterilized with gamma irradiation before undergoing in vitro analysis or being implanted in vivo. A scanning electron micrograph of the surface of one of the stents was taken to demonstrate its smooth homogeneous nature (Figure 2).

In vitro degradation studies and mechanical testing were performed on three 270° internal finger stents. Compression properties of the internal finger stents were determined with a mechanical tester (model Bionix 100; MTS Systems Corp, Eden Prairie, Minn). A 10-N maximum load cell was used with a crosshead speed of 1 mm/min. The internal finger stent was placed so that the axis of the stent lumen was perpendicular to the axis of the force applied. A dry weight and compressive modulus were determined before the stent was placed in 23 mL of Hanks balanced salt solution (HBSS; Gibco BRL, Grand Island, NY). After the stent was in buffer solution for 1 hour, it was taken out and a wet compressive modulus was measured. The modulus was then measured weekly following incubation of the stents in HBSS under static conditions at 37°C. If the stent cracked during mechanical testing, it was considered to have failed, and a modulus of zero was recorded for that week. One-milliliter aliquots were taken from the buffer solution each week to quantify polymer degradation. The amount of lactic and glycolic acid released during incubation in HBSS under static conditions at 37°C was measured over time. Lactic acid release was assayed enzymatically with lactate dehydrogenase using a kit from Sigma-Aldrich Chemical Co (St Louis, Mo). The release of glycolic acid was quantitated with a colorimetric assay, which involved decarboxylating glycolic acid in the presence of concentrated sulfuric acid to form formaldehyde, followed by reaction of formaldehyde with chromotropic acid to yield a colored product that could be quantitated spectrophotometrically. The amount of glycolic acid and lactic acid released into solution was plotted over time in buffer solution for each internal stent and averaged.

Each week the stents and their breakdown products were removed from the HBSS and dried in a vacuum oven overnight. A dry weight was recorded for each stent weekly. The stents were then placed back into their buffer solutions the next morning. The weights over time for each stent were then normalized to their initial dry weights and averaged.

Single-staged anterior tracheal reconstructions using homologous fascia lata grafts were performed in New Zealand white rabbits (preoperative weights, 2.5-4.0 kg) at the animal research facility at the Ann Arbor Veterans Affairs Medical Center (VAMC). Fascia lata grafts had been harvested postmortem from previous rabbits and sterilized in 2% glutaraldehyde solution for 15 minutes. The grafts were then stored in lactated Ringer solution at 4°C. The control arm of the study included 13 rabbits, and the experimental arm contained 12 rabbits. Each rabbit was anesthetized with ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (10 mg/kg) intramuscularly and given 1 dose of intramuscular chloramphenicol (50 mg/kg) preoperatively. The rabbits were allowed to breathe spontaneously throughout the procedure with no mechanical assistance given. A midline vertical skin incision was made in the neck exposing the cervical trachea, and a 3.5 × 15-mm vertical elliptical defect was created in the anterior tracheal wall starting approximately 1.0 cm below the cricoid using a standard template. A similar elliptical template was then used to cut a 6.5 × 18-mm graft from the previously prepared fascia lata for each animal. In the controls, the fascia lata was used to patch the anterior wall defect with a running chetis, foreign body reaction, granulation tissue formation, and stent migration. At our institution, endotracheal tube stenting with long-term ventilatory support is also sometimes used to maintain surgically reconstructed airways. However, infants and young children often require long-term paralysis and sedation with this type of stenting, which can increase the postoperative complication rates, prolong the child’s hospital stay, and increase the patient’s health care costs. The laryngeal complications of long-term intubation must also be considered. An absorbable internal tracheal stent could provide temporary rigidity and support to a tracheal reconstruction without causing long-term inflammatory and foreign body reactions, as seen with currently available stents, and allow the patient to function with a decannulated airway more quickly. Furthermore, because of its biodegradability, the stent would not have to be retrieved at a second procedure, thus lowering health care expenditures and avoiding the risks of a second anesthetic. Lochbihler et al were the first to describe using an absorbable stent intratracheally. They implanted stents made of unfusioned polyglactin 910 filaments in a homogeneous polydioxanone melt in the tracheas of rats. Korpela et al have reported on using self-reinforced poly-L-lactide helical spiral stents in a number of applications.

In this study a new biodegradable tracheal stent design was evaluated in a rabbit tracheal reconstruction model. This design may allow for improved mucociliary clearance in the trachea and minimize the amount of inflammation and foreign body reaction to the stent intraluminally. We describe the in vitro mechanical properties and degradative characteristics of the new internal “finger stent” design and demonstrate its use in a rabbit tracheal reconstruction model.

RESULTS

A total of 15 stents were used for this study. Three underwent in vitro testing while the other 12 were implanted in vivo. To characterize the mechanical properties of the stents in vitro, the dry compressive modulus...
The rabbits were housed at the Ann Arbor VAMC Research Facility. The animal use protocol was approved by the Institutional Animal Care and Use Committee of the Ann Arbor VAMC. Each rabbit was observed daily for any signs of respiratory distress. The rabbits were brought back for endoscopic evaluation of their reconstructed airways 5 times postoperatively (weeks 1 or 2, 3, 4, 5 or 6, 8 or 9, and 13). A telescopic bronchoscopy using a rigid 2.7-mm 0° telescope (Karl Storz Endoscopy, St Louis, Mo) was performed each time on the rabbits. The examinations were videotaped, and measurements of airway diameter were made at a distal nonreconstructed site and at the narrowest point of the reconstructed airway using a balloon catheter technique described by Othersen.17 The airway measurements in the internally stented animals were not done until the stents had degraded enough to permit an internal measurement. The cross-sectional area and percentage stenosis were then calculated for each rabbit based on these measurements. The average percent stenosis for each group was then determined for each set of bronchoscopic examinations.

The rabbits were humanely killed 3 months postoperatively (at 13 weeks) with a lethal dose of intravenous potassium chloride after a final bronchoscopic examination and airway measurement were performed. Their tracheas were harvested and polyvinylsiloxane (PVS) casts were made of their lumens. If the rabbits died prematurely, a postmortem bronchoscopic examination of their airway and airway measurements were performed before their tracheas were harvested. The tracheas were formalin fixed, embedded in paraffin, and sectioned into 5-mm cassettes with a microtome. Sixteen slides (4 from each cassette) were prepared at 1-mm intervals along the length of the reconstructed segment. The slides were stained with hematoxylin-eosin and evaluated under a light microscope. Assessment of qualitative features, including new tissue growth and inflammatory changes, were made for both the control and internal stent groups.

Using the PVS impressions, the average stenosis for a portion of the reconstructed tracheal segment was determined by a volume displacement technique. A fixed portion of the reconstructed tracheal segment was cut out of the cast centered on the most stenotic point, and an equal length portion of normal trachea was cut distally for each rabbit. Each of the cut sections was then submerged in deionized water in an inverted graduated glass syringe, and the amount of water displacement was measured. Based on these volume displacement measurements, the average percent stenosis across the entire reconstructed segment was determined. These results were then averaged for both the control and internally stented animals. Next the PVS cast of the reconstructed segment was sectioned at its most stenotic point and an ink impression of the cross-sectional area was made on paper. This was also done for a nonreconstructed tracheal section distal to the reconstruction site. The 2 ink impressions were then scanned into a computer and the cross-sectional areas were measured in pixels using Scion Image 1.60a (Scion Corporation, Frederick, Md). The percent stenosis at this most stenotic point was then calculated for each rabbit and averaged for both the control and internal stent groups.

Comparisons between the control group and the internal stent group were made for the average percent stenosis, as measured by the balloon catheter method at each bronchoscopy, and as measured by the volume displacement method using the PVS casts. The same comparison was also made between the groups for the PVS cast measurements looking at the most stenotic point of the reconstructed segment. To determine whether the differences seen between the groups were statistically significant, a non-paired 1-tailed t test (a Student t test if the SDs were essentially equal and an Alternate Welch t test for unequal SDs) was performed. Differences at P<.05 were considered significant.

FIGURE 6, once the stents were degraded, were also done at the time of bronchoscopy using a balloon catheter. The attrition rate for the control group was 23% (3/13). Rabbits 3, 7, and 9 all died before completion of the study. Rabbit 3 developed stridor on postoperative day 3 and died on postoperative day 14 of airway collapse secondary to an anterior paratracheal abscess. Rabbit 7 died of an anesthetic overdose, and rabbit 9 died the day after a routine bronchoscopy (postoperative day 36), most likely from swelling in an already compromised airway. It had been stridorous for 2 days prior to the bronchoscopy. Rabbits 3 and 9 had stenoses greater than 40%. Only 1 rabbit (rabbit 8) survived with a stenosis greater than 40%. Intermittent stridor was also noted in rabbits 2 (beginning at 3 weeks), 4 (at 7 weeks), and 8 (at 2 weeks), giving an overall stridor prevalence of 38% for the control group. In 8 (62%) of the 13 control rabbits, there was no evidence of stridor or respiratory distress.

None of the internally stented rabbits were measured at the first 2 bronchoscopy dates (weeks 2 and 4).
since their internal stents were still present. By the third bronchoscopy (week 6), 7 of the animals could be measured. At the fourth and fifth bronchoscopy (weeks 9 and 13, respectively) all of the internally stented animals that were still alive were able to be measured. Both the attrition rate and stridor prevalence in the internal stent group were 17% (2 of 12). Rabbits 65 and 67 died before the completion of the study. Rabbit 67 developed stridor 3 weeks postoperatively and died of airway collapse 42 days after operation. At the second bronchoscopy (week 4), the stent was partially degraded and collapsed into the tracheal lumen. The stent fragments were removed, but the airway reconstruction subsequently collapsed at that point. Rabbit 65 died the morning after routine bronchoscopy (postoperative day 64), most likely from post-bronchoscopy swelling. It had not had any stridor prior to bronchoscopy and had an average stenosis of less than 30%. One other internally stented animal developed stridor (rabbit 70) during the study but survived the entire 3 months. The other internally stented rabbits showed no change in their clinical course as the stents de-
Comparison of Final Average Percent Stenosis Between Control and Internal Finger Stent Groups by Method*

<table>
<thead>
<tr>
<th>Group</th>
<th>Rabbit No.</th>
<th>Volume Displacement Method Using PVS Casts</th>
<th>Most Stenotic Point Method Using PVS Casts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>61, 62, 68, 70, and 72</td>
<td>34</td>
<td>36</td>
</tr>
<tr>
<td>Stented</td>
<td>63, 64, 66, 69, and 71</td>
<td>23</td>
<td>33</td>
</tr>
</tbody>
</table>

*PVS indicates polyvinylsiloxane.

graded. Gross migration of the stents was not seen at any of the bronchoscopies. However, it was noted early on in the clinical course (postoperative weeks 1 and 2) that the stents could be moved by the endoscope if the surgeon was not careful in assessing the airway. At each bronchoscopic examination, visualization down to the carina and takeoff of the mainstem bronchi was achieved, and at no point in the study was stent debris or mucosal sloughing noted in the distal airway of any animal. When the stents became fragmented, the rabbits were generally able to expel the fragments out of the airway. In only one rabbit did the fragments become aggregated locally and cause obstruction resulting in the need for endoscopic removal (rabbit 67).

There were no significant differences in the average percent stenosis of the reconstructed airways between the 2 groups as measured by the balloon catheter until week 13. At this final bronchoscopic examination, the internally stented rabbits had a significantly smaller average percent stenosis (35%) than the control rabbits (47%) (P<.05) (Table). After 3 months, the rabbits were killed, their tracheas were harvested, and PVS casts were made of their internal lumens. Using the volume displacement measurements, the internally stented rabbits had a significantly smaller average stenosis across their reconstructed segments (23%) than the control rabbits (34%) (P<.05). When the average stenosis was measured at the most stenotic point only, as opposed to being averaged across the entire segment length, there was no statistically significant difference between the 2 groups (controls, 36%; internal stents 35%).

Sectioning and staining of the harvested tracheas allowed for microscopic assessment of the reconstructed segments in both groups. The extent of subepithelial edema, acute inflammation, chondrification, ossification, and granulomatous inflammation was assessed. In the control animals, the anterior reconstruction site was placed externally. Subepithelial edema was present in 5 (42%) of 12 control animals and small foci of acute inflammation in 6 (50%) of 12 animals. Complete circumferential periluminal edema and extensive acute inflammation was noted in rabbit 9, which had died prematurely of postbronchoscopy swelling in an already compromised airway.

In the rabbits with internal stents, fibrosis bridged the gap between the lateral cartilage remnants resulting in a more circular cross-sectional configuration of the trachea (Figure 7, right). Fascia lata grafts were viable and tended to occupy a position anterior to the fibrosis bridging the gap. Chondrification was present in all internally stented animals but only rare small foci of ossification were present in 3 of 12 animals. In contrast to the control animals, the new cartilage growth was contributing to the newly reconstructed anterior tracheal wall instead of being displaced externally. Subepithelial edema was less than that observed in the control rabbits (2 of 12 animals, 17%) and foci of acute inflammation were noted in 5 (42%) of 12 animals. Granulomatous inflammation was similar to that seen in control animals. While lumenal reepithelialization was generally complete in all animals, small fragments of stent material were often evident within the tracheal lumen.

This study introduced a new “finger-like” biodegradable internal tracheal stent with well-characterized in vitro

![Figure 6](chart.pdf) Average stenosis for each internal stent rabbit as measured by the balloon catheter method during routine bronchoscopic examinations (n=3 measurements for rabbits 61, 62, 68, 70, and 72, and n=2 measurements for rabbits 63, 64, 66, 69, and 71; rabbit 65 [n=2] and rabbit 67 [n=1] died prematurely).

![Figure 7](histo.png) Left, Histological cross section of a reconstructed trachea from a control rabbit. Right, Histological cross section of a reconstructed trachea from an internally stented rabbit.
compressive mechanical properties and degradative characteristics. Its use in a rabbit tracheal reconstruction model was also demonstrated. A statistically significant decrease in the average stenosis across the newly reconstructed segments was seen in the internally stented rabbits compared with the control rabbits. A statistically significant decrease in stenosis was also measured at the most stenotic point of the reconstructions when using a balloon catheter technique; however, a nonappreciable difference between the 2 groups was noted at the most stenotic point when using the PVS tracheal cast measurements. Both the stridor rate and death rate were also lower in the internal stent group. Overall, the presence of the internal finger stent had a positive effect on airway size in the experimental group.

The in vitro degradation of the polymers used in the fabrication of the internal stents has been well described. Poly(D,l-lactide-co-glycolide) degrades by simple hydrolysis, and as expected, the glycolic acid component of the internal stents degraded more rapidly than the lactic acid component. The internal stents began to lose significant mass after 5 weeks and were nearly completely eroded by 15 weeks. However, the in vivo degradation of the internal stents was more complex. A combination of degradation and airway expulsion of stent fragments by the rabbits resulted in the early disappearance of the stents. By 6 weeks, 7 of 12 internally stented rabbits could be measured internally since their stents were gone, and by 9 weeks, all of the stents had disappeared. Thus, the degradation rate of the stents was probably quicker in vivo than in vitro, but the expulsion of eroded fragments most likely contributed to the shorter elimination time seen in vivo.

The internal finger stents performed well in vivo. The stridor rate and overall mortality rate for our internal stent group were both 17%. The control group had a stridor rate of 38% and an overall mortality rate of 23%. If the one animal in the control group that died of an anesthetic overdose is excluded, then the attrition rate of the control group equaled that of the internal stent group (17%). The decrease in stridor rate seen in the internally stented rabbits indicates that the stents aided in preventing collapse of the reconstructed anterior wall without causing new problems due to inflammatory or foreign body reaction to the stent. This is in contrast to what Lochbihler et al. reported with their internal polydioxanone suture airway stents. In their study, 14.3% of the rats died during the first week after implantation due to inflammatory granulomatous obstruction of the stented airway segments. The minimal inflammatory response seen with our stents might have been due to the type of polymer used or might have been the result of the patented fabrication process.

The finger stent design used in this study also compared favorably with prior studies published by Korpela and his colleagues in Finland, who used an internal helical coil design for their biodegradable tracheal stent. In one study, they implanted coiled poly-L-lactide stents into the normal cervical tracheas of rabbits through a transverse airway incision. They had a stridor rate of 22.2% but no deaths. In another study, which more closely mimics our use of biodegradable stents in surgically altered airways, they implanted their coil design stent into rabbits that had experimentally created tracheal stenosis. Four (36%) of 11 rabbits died before the completion of the study. Two of their rabbits died as a result of stent fragments occluding the airway.

In this study, only one of the internally stented rabbits was compromised from stent fragments occluding the airway. Although the fragments were easily removed, the rabbit died 14 days later. Based on our experience with this animal and on our bronchoscopic observations from the other animals, it is apparent that stent breakdown leaves fragments of various sizes either free within the airway or partially adherent to the tracheal wall. The majority of the rabbits were able to expectorate these fragments without any apparent difficulty. This most likely was due to the 270° finger design. There is a lower mass of stent material to degrade with this design than with the 360° coil design, and with the posterior gap, mucociliary clearance can proceed unimpeded and clearance of any small stent fragments that fall into the airway may be facilitated. If any part of the stent becomes mucosalized, expectoration of the fragments or endoscopic removal becomes more difficult. However, tighter adhesion to the luminal wall and resulting mucosalization may be preferred because it negates the problem of free-floating fragments in the airway. In the present study there was little mucosalization of the finger stents because of inconsistent contact of the stents with the tracheal wall. Thus, the issues of stent adherence and mucosalization and their impact on endoscopic removal remain unresolved.

The discrepancy seen in the control rabbits between their final balloon catheter measurements and their PVS cast measurements at the most stenotic point of their reconstructed segments can most likely be explained by measurement bias and the irregularly shaped airways that were encountered. All of the control airways had a teardrop shape or slitlike elliptical shape. It was difficult to measure these airways with a spherical balloon catheter without dilating the lateral walls of the trachea, especially later in the postoperative course as scarring and fibrosis set in. Thus, the balloon was probably underexpanded at times when the control animals were measured, leading to a higher measured stenosis (47% average stenosis by balloon measurements vs 36% by PVS cast measurements). The internally stented animals, in contrast, had more circular stenoses, and thus were more accurately measured with the balloon catheter method. This is obviously a disadvantage to using Othersen’s method to measure airway stenoses. Othersen’s method does prove helpful in estimating the size of irregularly shaped airways and is invaluable in following circular airway size in living patients and animal subjects in a safe and expedient manner. We believe that the PVS cast method of measurement is more accurate when taking into account irregularly shaped airways, but, unfortunately, it can only be performed post mortem.

The strength and mechanical properties of the internal finger stents may need to be maintained longer than 4 weeks, if the improvement in lumen patency is to be seen along the entire length of the reconstructed segment. When the internal finger stents broke, they tended to fail at just one point along the reconstruction site. This was demonstrated by the PVS cast measurements that were done at the most stenotic point of the reconstructed air-
ways, which showed essentially no difference between the 2 groups. This was in contrast to the lower average percent stenosis, which was statistically significant, that was seen in the internal stent group when the entire reconstructed segment was measured. Furthermore, the in vitro data demonstrated that the finger stents tended to break after just 4 weeks in buffer solution. A greater proportion of the stent composition may need to be made of polyactic acid to strengthen the stent under compressional stress. Korpela et al13-15 have previously utilized stents fabricated from pure poly-L-lactide. However, the disadvantage to increasing the polyactic acid component in the stents is that the time to complete degradation would also increase, thus prolonging the potential for any inflammatory or foreign body reaction to the stent. Fortunately, our histologic and bronchoscopic examinations showed little evidence of inflammatory reaction to the stent; thus, this should be a promising way in which to fabricate future stent designs.

Another approach to making a stronger reconstructed airway is to accelerate regenration of the native tracheal tissues to obviate the need for a stent to supply mechanical support for longer than 4 weeks. The histologic results demonstrated areas of new cartilage growth around the fascia lata graft in both groups. By cross-linking peptides to the biodegradable polymer scaffold, a controlled and sustained release of growth factors could be achieved to promote accelerated cartilage growth across the reconstructed segment. The growth of new cartilage across the reconstructed segment would ultimately provide the greatest stability to a newly reconstructed airway.

Biodegradable internal tracheal stenting is a promising approach to augmenting tracheal reconstructions. Our results suggest that internal “finger-like” biodegradable stents degrade in a predictable fashion and have a statistically significant effect in augmenting anterior tracheal reconstructions with fascia lata in rabbits. The 270° finger design also appears to provide some advantages over a 360° coil design that has been used previously. By increasing the polyactic acid component of the stents, the duration of the mechanical properties of the stent should increase without compromising on the advantages their biodegradation brings. Moreover, the use of biodegradable internal stents in tracheal tissue engineering applications may be a promising extension of this study.

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