A Novel Bioabsorbable Drug-Eluting Tracheal Stent

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**Objectives/Hypothesis:** Currently available silicone and metallic stents for tracheal stenosis are associated with problems of granulations, mucous trapping, and difficult removals. Our aim was to develop a novel bioabsorbable tracheal stent with mitomycin C (MMC) drug elution to circumvent such problems.

**Study Design:** A randomized animal study.

**Methods:** Twenty-five rabbits were randomly assigned into five test groups: 1) controls (without stent), 2) silicone tubular stents (commercially available currently); 3) bioabsorbable helical stents; 4) bioabsorbable tubular stents; and 5) bioabsorbable tubular stents with MMC. Weekly tracheal endoscopy to document granulation, mucus plugging, and extent of tracheal stenosis was performed for 12 weeks. One rabbit was euthanized every 3 weeks for histological analysis of the trachea. In vitro MMC-release profiles in conditions mimicking tracheal conditions were studied.

**Results:** The bioabsorbable tubular stents with 0.1 mg MMC drug elution performed the best, with the least mucus trapping and airway obstruction due to tracheal stenosis. Tracheal stenosis was most significant for the bioabsorbable helical stents, followed by the control group without stent, the group of bioabsorbable tubular stents, and then the silicone stents. After 12 weeks, tracheal stenosis for the bioabsorbable tubular stents with MMC was only half that of the silicone stents.

**Conclusions:** This study reports on the development of a novel bioabsorbable tracheal stent with sustained MMC drug elution for preventing tracheal stenosis. Further studies are warranted to optimize stent design and drug dosage.

**Key Words:** Tracheal stent, tracheal stenosis, mitomycin C.

**Levels of Evidence:** 2c.

Laryngoscope, 121:2234–2239, 2011

**INTRODUCTION**

Tracheal airway stenosis results from prolonged endotracheal intubation, tracheotomy, trauma, infections, tumor or tumor-related treatment, and congenital disorders. Surgical intervention may be needed to re-establish a patent airway, with insertion of stents to prevent restenosis. Currently available stents include silicone stents, metallic stents, and stents that combine a silicone or synthetic outer coating with metal hoops or mesh. Silicone stents like the Dumon, Montgomery, and Hood stents are the most widely used clinically. They are well tolerated, removable, and flexible. However, they impair physiologic mucociliary function, trapping airway secretions and mucous plugs, and have associated reactionary granulations at the proximal and distal ends, with risks of airway obstruction. Silicone stents also have thick walls that narrow the trachea lumen patency, limiting their use in neonates and younger children unless a tracheotomy is also performed. Metallic stents can be inserted endotracheally without open surgery, have less trapping of secretions, and have thinner walls. However, metallic stents are difficult to remove once they are mucosalized over by epithelium. Metallic stents may sometimes also fragment, extrude, and penetrate into neighboring structures like the esophagus and large neck vessels.

Tracheal stents made of bioabsorbable polymers may be ideal as they can provide temporary rigidity before self-absorption, and may not need another general anesthesia for removal. We hypothesize that bioabsorbable stents made of biocompatible materials with specially designed cutouts within the stents may be the most ideal. These stents can be very thin-walled, and cutouts may allow mucosalization within the stented area of the trachea, improve mucociliary clearance of secretions, and reduce the reactionary granulations and subsequent tracheal stenosis at proximal and distal ends of the stents. If there is no significant mucus plugging within the stents, tracheotomy may also not be needed for patients with a tracheal stent.

Bioabsorbable stents can allow sustained drug elution to prevent restenosis. Drug-eluting stents have provided good drug delivery platforms for reducing restenosis in cardiology, urology, esophageal, and gastroenterology fields.
Zilberman et al. undertook in vitro studies without animal models, and floated their poly-L-lactide films on sterile water at 37°C to better simulate the unique conditions of both water and air interfaces in the trachea. The results showed good mechanical properties and almost near-linear release profiles for dexamethasone. Mitomycin C (MMC) is an antimitotic drug that is widely used as a treatment for several types of cancers. It was first used as an ophthalmic agent to treat glaucoma. MMC inhibits DNA synthesis and cellular RNA and protein synthesis are also suppressed.

The aim of this study was to develop and investigate the effects and efficacy of a novel bioabsorbable tracheal stent, with and without MMC elution, for the prevention of tracheal stenosis. To the best of our knowledge, this is the first study to determine if a drug-eluting bioabsorbable tracheal stent can prevent tracheal stenosis in an animal model. This is also the first study on the delivery of MMC in a sustained-release method via a drug-eluting stent.

MATERIALS AND METHODS

In Vitro MMC Release Samples and Analysis

In vitro MMC release studies were performed to simulate MMC release from the drug-loaded tubular stents. Films incorporating MMC were prepared by solution casting. These films (n = 3) containing MMC at 0.1 mg/film were immersed in 2 mL of distilled water in glass vials to ensure sink conditions and placed in a 37°C incubator, with the medium changed weekly. Drug stability and release were studied using reversed-phase high performance liquid chromatography (HPLC) and measured at a wavelength of 365 nm. After the last time point, extraction of any residual MMC in the films was performed by dissolving all films completely in an organic solvent (tetrahydrofuran) and analyzed by HPLC. Release profiles were normalized based on the total loading determined in this manner.

Stent Fabrication

Two stent designs were used in this study: helical and tubular (Fig. 1). Both were fabricated based on the bioabsorbable copolymer, poly(L-lactide-co-ε-caprolactone) (PLLA-PCL) 70/30, (Purac Biochem BV, Gorinchem, The Netherlands). Glycerol (Sigma-Aldrich Inc., St. Louis, MO), was added to PLLA-PCL at 10% by weight to increase water uptake into the copolymer and reduce degradation time to 6 weeks to 3 months for a tracheal stenosis application. MMC was purchased from Hande Industry and Trade Holdings Limited (Shenzhen, China), and its final dosage was optimized to 0.1 mg per stent. A provisional patent application (US Patent Application No. 61/454,858) has been submitted to cover these compositions and drug loadings.

Sizes of stents chosen to be studied were those that could fit a pediatric tracheal airway. Silicone stents used were tubes with 1 mm wall thickness, 6 mm outer diameter (OD) and 10 mm length. All fabricated stents had 0.25 mm wall thickness, 6 mm ± 0.2 mm OD and 10 mm length. Helical-shaped stents were fabricated from PLLA-PCL + 10% glycerol strips. Tubular-shaped stents had 12 rectangular holes cut and distributed throughout each PLLA-PCL + 10% glycerol film. For the tubular stent with MMC, MMC was added to the polymer solutions, homogenized, and casted.

Animal Study

Both IACUC (#070/07(A1/08) and OSHE (OSHE/RA/3/04/FOM-466) approvals were obtained. All surgical procedures were performed by the same surgeon in an aseptic manner. Five groups of five New Zealand White rabbits in each group were studied, each weighing 3.5 to 4.0 kg. Trachea stenosis was created in all groups using unipolar diathermy. The five groups were 1) control 1—without stent; 2) control 2—commercially available silicone tubular-shaped stent; 3) bioabsorbable helical-shaped stent; 4) bioabsorbable tubular-shaped stent; and 5) bioabsorbable tubular-shaped stent with MMC.

Surgical Techniques

Each rabbit received ketamine hydrochloride (7.5 mg/kg) and xylazine (10 mg/kg) intramuscularly for general anesthesia and were spontaneously breathing during the 10 minute surgery. The trachea was exposed through a midline vertical skin incision in the neck, strap muscles were retracted laterally, and the midline anterior tracheal wall exposed. A midline tracheal incision was made onto the anterior trachea wall between the third and seventh tracheal rings. Unipolar diathermy at 35 W was used to create mucosa injury and stenosis circumferentially between the 4th to 6th rings. The stents to be studied were implanted between the 4th and 6th rings. To prevent the stents from sludging, 5-0 nylon suture was used to place two sutures from the stent to the anterior trachea wall.

Each rabbit was observed daily for respiratory distress and well-being. Rabbits with body weight loss of more than 20%, with respiratory distress, or anorexia were euthanized. Their Airways were evaluated weekly with rigid 2.9 mm diameter 0° endoscopes (Karl Storz Endoscopy, St. Louis, MO). Endoscopic examinations were digitally recorded and video frames were paused at a fixed distance from the stent. From the image on the screen, a measuring tape was used to measure the most stenosed distance from this two-dimensional cross-section, and the percentage of tracheal stenosis was calculated by assuming all stenoses were concentric. Two independent reviewers reviewed the videos and measurements to score and grade the degree of stenosis, with similar inter-rater and intra-rater concurrence of the severity of tracheal stenosis. For this study, the results of the severity of tracheal stenosis were reported in terms of percentage stenosis of the cross-sectional area of the trachea instead of the Cotton-Myer scale as described by Eliashar. This allowed more differentiation between different study groups, compared to the four grades of...
the Cotton-Myer scale. All percentage stenosis reported were the average values from both reviewers and included all surviving rabbits in each group at each particular time point.

**Histology**

One rabbit from each group was euthanized every 3 weeks after endoscopic examination. Tracheal tissues were collected immediately after euthanasia and fixed in 10% neutral-buffered formalin for a minimum of 48 hours. Tissues were trimmed, processed routinely for histology, and embedded in paraffin. Sections that were 5-μm thick were cut and stained with hematoxylin and eosin for morphologic evaluation by light microscopy by a veterinary pathologist.

**RESULTS**

**In Vitro MMC Release Study**

In vitro MMC release studies were performed to correlate the in vivo results from implanted tubular stents with MMC. As the implanted stents were subjected to a relatively harsh environment in the rabbits’ tracheas with continuous mucus flow and tracheal movement, the in vitro release samples were immersed in water to achieve a release profile mimicking that expected in vivo.

The equation below was used to obtain the kinetic data:

\[ \frac{M_t}{M_\infty} = kt^n \]

where \( M_t/M_\infty \) is the fraction or percentage of total drug \( (M_\infty) \) released at time \( t \), \( k \) is a constant depending on the conditions of the system, and \( n \) is the exponent that describes the diffusional release kinetic mechanism.\(^{10}\)

From the results obtained (Fig. 2), a total of only about 33% of efficacious MMC loaded into the bioabsorbable films was released into the media in a 12-week period. The diffusional exponent \( n \) was 0.3108 and a regression coefficient close to 1 was achieved, indicating the applicability of the equation.

**In Vivo Animal Studies**

All 25 rabbits recovered well after surgical implantation of the stents. Five rabbits required euthanasia before their scheduled sacrifice due to respiratory distress. One rabbit each in control group 1, control group 2, and group 3 died due to anesthesia drug overdose during endoscopic examinations between week 5 and 7 following insertion of the stents. They were otherwise well before anesthesia, and deaths were not stent related. For subsequent rabbits, only one third of the dosage of anesthesia for implantation of the stents was used during the endoscopics, and early reversal from anesthesia was done. There were no further anesthesia-related deaths.

Apart from two instances of early degradation of the bioabsorbable stents in groups 4 and 5, all other implanted stents remained intact and unresorbed up until the scheduled sacrifice or euthanasia time points.

**Control Group 1—Without Stent**

Unipolar diathermy at 35 W was used to induce stenosis in the tracheas of the rabbits. Stable tracheal stenosis narrowing the lumen cross-sectional area by about 75% was achieved. Stenosis was significant by 3 weeks and stable by 6 weeks following diathermy injury (Fig. 3A).

**Control Group 2—Commercial Silicone Tubular-Shaped Stent**

Commercially available silicone stents were used to stent the trachea after diathermy injury. One rabbit developed severe postintubation stenosis at the tube cuff site at 3 weeks, and this rabbit was euthanized. The remaining four rabbits developed cloudy, thick, adherent mucus within their stents, which resulted in respiratory distress (Fig. 3B).

**Group 3—Bioabsorbable Helical-Shaped Stent**

The bioabsorbable helical-shaped stents caused profuse tissue reaction in the trachea to develop between the nonstented areas of the trachea between the helices of the stent. Among all of the groups, it had the most severe stenosis and mucus trapping in the tracheal lumen (Fig. 3C). Two rabbits had to be euthanized at 4 and 6 weeks, and no rabbit survived beyond 6 weeks. In all the other groups, at least one rabbit in each group survived up to 12 weeks, and no rabbit had to be euthanized due to excessive tracheal stenosis.

**Group 4—Bioabsorbable Tubular-Shaped Stent**

The tubular stents unwound to fit the diameters of the tracheal lumens after insertion. This group had less mucus trapping and airway narrowing compared to the helical bioabsorbable stents, and the outcomes were similar to that for the commercial silicone tubular-shaped stent group during the first 6 weeks after stenting (Fig. 3D). A rabbit in this group died at the 8th week due to obstruction of the trachea by degraded stent fragments.

**Group 5—Bioabsorbable Tubular-Shaped Stent With MMC**

Among all groups, this group had the least calculated tracheal stenosis (Fig. 4). Sustained release of MMC at approximately 200 μg/day from these stents showed enhanced efficacy in inhibiting excessive tissue...
growth. At 11 weeks, one stent degraded into two parts, and was coughed out by the rabbit. This resulted in excessive tissue growth and progressive stenosis with blockage of 80% of the tracheal lumen 1 week later.

**Extent of Tracheal Lumen Stenosis in All Five Groups**

Figure 5 shows the extent of tracheal lumen stenosis for the five groups over the follow-up duration of 12 weeks. These values were the average severity of tracheal stenosis observed based on all surviving rabbits within a group at that particular week of endoscopy. Tracheal lumen stenosis was most significant in the bioabsorbable helical stents, followed by the group without stents, the bioabsorbable tubular stents, and finally the silicone stents. After 12 weeks, tracheal stenosis for the bioabsorbable tubular stents with MMC was half that of the silicone stents, averaging 10% and 20% tracheal stenosis respectively.

**Histology of Tracheas Harvested After Euthanasia**

The light microscopic changes seen were similar across all five groups and were consistent with injury repair and healing. Mild to moderate submucosal edema, subacute to chronic inflammatory response, granulation tissue formation, and mucosal regeneration were present to some degree in all groups.

**DISCUSSION**

The rabbit animal model was chosen here as its airway diameter is very similar to that of a neonate and young pediatric patient. In this group of patients, the risks of airway obstruction are most significant, making wall thickness, mucociliary clearance, and risks of granulations and stenosis of tracheal stents even more important. Furthermore, follow-up endoscopy can be performed in a manner similar to that for human patients. Tracheal stenosis was also created by controlled diathermy heat injury to simulate the conditions of the injured trachea that would benefit from stenting in real life, rather than applying the stents to a normal trachea.

The results of the study are summarized here. Currently available commercial silicone stents are complete tubular stents without cutout holes in the stent. Therefore, granulations and stenosis occur only at the proximal and distal ends of the stents. The silicone stents resulted in tracheal stenosis ranging between 10% to 45%. The bioabsorbable helical stents had the most severe tracheal stenosis, ranging from 22.5% to
99% stenosis. This was due to the nonstented areas of injured trachea between the helical turns reacting with profuse granulations and fixed stenosis eventually. For our bioabsorbable tubular stents, we placed cutout holes distributed evenly throughout the stent to allow preservation of mucosa even within the stented area of the trachea. This preserves healthy tracheal mucociliary activity, improves mucus clearance and tracheal patency. However, for the group with bioabsorbable tubular stents without MMC elution, the cutout holes do allow some granulations and stenosis to surface within the stented area of the injured trachea. The group with the novel bioabsorbable tubular stent with MMC-elution performed the best among all groups of stents. It had the least tracheal stenosis at 10% and the least mucus trapping within the stents at 12 weeks. This is likely contributed by the MMC elution inhibiting tracheal granulations and stenosis, both within the cutout areas of the tubular stents and also at the proximal and distal ends of the stents.

Previous studies of bioabsorbable tracheal stents of various designs in rat or rabbit models involved mainly polymeric materials of polydioxanone, poly(L-lactide) (PLLA) and poly(D,L-lactide-co-glycolide) (PLGA). In these studies, the stents were immersed in buffer solutions while studying their degradation profiles. The main problems were excessive granulations, lack of mucosalization over the stent walls, and stent expulsion due to degradation. During in vitro testing, fragmentation and significant mass loss occurred suddenly for such polymers. To address these problems, we investigated several other bioabsorbable polymer candidates, some with plasticizers added during fabrication of a batch of tracheal stents for an initial feasibility study. Stents fabricated from PLLA-poly-ε-caprolactone (PCL) and PLGA with varying amounts of plasticizers, and PCL were implanted in a pilot group of rabbits. PLLA-PCL with 10% by weight of glycerol was the best tolerated material. It maintained its structural integrity throughout the duration of study, and its inherent softness did not induce excessive granulation and scar tissue growth in the trachea. PLLA-PCL + 10% by weight glycerol was therefore used as the main blend for all bioabsorbable stents fabricated and implanted in this study.

This is the first study that investigated the outcomes of a controlled amount of MMC application over a few weeks via drug elution from a stent. Rahbar et al. reported that topical MMC effectively prevented scar formation in the aerodigestive tract. The effect of MMC for inhibition of tracheal stenosis is still not completely clear. Smith et al. reported that restenosis and delayed symptom recurrence were similar after endoscopic dilation with or without MMC. Shvidler et al. and Hartnick et al. did not observe significant differences between control and MMC treatment groups. However, these studies were conducted with MMC being only topically applied directly to the trachea for a few minutes and without the application of stents. In all previous studies, MMC could only be applied topically for 1 to 5 minutes to avoid blocking the airway during applications. Although the topical application held 0.1 to 1 mg/mL of MMC, the actual amount delivered this way is unknown.

From the 12-week in vitro cumulative release profile of films with 0.1 mg MMC (Fig. 2), it appeared that MMC was released via a diffusional release mechanism. The exponent of the diffusion equation is ~0.3, which indicates some deviation from classical Fickian diffusion from a slab geometry (expected n = 0.5). Nevertheless, the mechanism is largely diffusion-controlled with about 33% released over 12 weeks. Assuming delivery from the stent to the trachea was unidirectional and no degradation of MMC occurred, a low dosage of 0.0165 mg/stent (0.33 x 0.1 mg x 0.5) would have been sufficient to prevent tracheal stenosis if the stent degrades completely in the 12-week period of the study. The release of MMC is expected to reach completion when the bioabsorbable polymer begins to degrade significantly at a later stage, changing the release kinetics from diffusion to a more polymer degradation-controlled profile.

The limitations in this preliminary study include the small number of rabbits at five per group, which make statistical analysis less useful, the deaths of three rabbits due to anesthesia in the early stage of the study, a follow-up of 4 to 6 months following implantation of stents precluding study following explantation of stents, and relatively limited histology studies. This preliminary study has, however, given us sufficient data to request funding for a follow-up study with modifications to address the limitations of this study.

In an ongoing follow-up study, we will further identify the optimal dose for MMC drug elution and determine the optimal timing of stent degradation. We will further improve on stent design to ensure sufficient mucosalization over the cutout holes in the stent, as that not only improves mucus clearance, but also allows stent degradation to occur with optimal safety without obstruction of the tracheal airflow.

CONCLUSION

Three types of bioabsorbable stents and designs (helical, tubular, and tubular with MMC elution) were compared to the commercially available silicone tubular stent for the prevention of tracheal stenosis. Our novel bioabsorbable tubular stent of PLLA-PCL polymer with MMC drug elution resulted in the least tracheal stenosis and mucus trapping at 12 weeks following stenting of injured rabbit tracheas averaging 6 mm in diameter. This preliminary study suggests that the sustained release of MMC via a bioabsorbable stent in the trachea can be further studied to determine if it can perform better than currently available stents for the prevention of tracheal stenosis.

BIBLIOGRAPHY


