Experimental study on an airway prosthesis made of a new metastable β -type titanium alloy

Aixing Tan, MD,^a Shiying Cheng, MD,^a Pengcheng Cui, MD,^a Pengfei Gao, MD,^a Jiasheng Luo, MD,^a Chunshu Fang, MD,^a and Zuoqing Zhao, MD^b

Objective: Surgical treatment of a subglottic stenosis can lead to a cricoid cartilage resection, cervical trachea resection, or both that has to be reconstructed with an artificial prosthesis. However, there is at present no standard treatment in the numerous reconstruction procedures, and many prostheses encounter inflammatory rejection and mechanical constraint problems. A porous, metastable β -type titanium alloy prosthesis was developed to improve subglottic rehabilitation.

Methods: We have designed a porous type of airway prosthesis made of porous metastable β -type titanium alloy. We used this prosthesis (20 mm in length) to reconstruct the cricoid cartilage and the first tracheal ring in 10 mongrel dogs and evaluated its efficacy.

Results: One dog died of an accident with anesthesia at 1 week, 1 dog died of pneumonia about 1 month after the operation, and 6 dogs were killed between the third and eighth months after the operation. At death, all the prostheses had become completely incorporated into the host tissue. Endoscopic examination showed no airway obstruction for a postoperative period of 3 to 8 months in 8 dogs. Granulation was observed in 4 cases, and the porous, metastable β -type titanium alloy plate was exposed in 1 case, but these dogs were asymptomatic. Light microscopic and electron microscopic analysis showed a normal mucous membrane growth on the surface of the prosthesis and no visible granulation tissue in the reconstructed cricoid and tracheal cavity.

Conclusions: This airway prosthesis provided good results in canine cricotracheal reconstruction, and this study presents the possibility of successful reconstruction of the cricoid and trachea with epithelial regeneration. (J Thorac Cardiovasc Surg 2011;141:888-94)

In clinical practice many different methods of management of subglottic stenosis of a variety of causes have been described. Conservative treatment methods, such as corticosteroid injection and balloon dilation, have been performed poorly in the majority of cases, and open surgical intervention is considered the standard of care for hard and fibrous subglottic stenosis.^{1,2} A treatment method of subglottic stenosis should be chosen according to the severity of stenosis. Mild stenosis can heal spontaneously.³ For moderate stenosis requiring surgical intervention, the first-choice method of treatment is laryngotracheal reconstruction, a small procedure in which the cricoid cartilage is split and expanded with a graft. For severe stenosis with or without vocal fold involvement requiring surgical intervention, the best method of treatment is laryngotracheal resection.⁴ Different materials and techniques have been used to deal with this effect.⁵⁻⁹ However, information from a variety of

reports indicates that there has been no standard treatment in the numerous reconstruction procedures thus far, which reflects that the management of laryngotracheal stenosis remains a challenge for the otolaryngologist.

Titanium and titanium alloys are widely used for many biomedical applications because of their low density, excellent biocompatibility, corrosion resistance, and mechanical properties.^{10,11} In our study we selected the new β -type titanium alloys (designated hereafter as TLM) produced by the Northwest Institute for Nonferrous Metal Research (Xi'an, China). Having good mechanical strength and lower modulus of elasticity, they are considered to be the most attractive metallic materials in biomedical applications.¹²

The purpose of the current study was to evaluate the efficacy of cricotracheal reconstruction with a TLM in a canine model for the treatment of subglottic stenosis. Biocompatibility tests of the used materials are very important for a successful implantation. The material must have low or no toxicity biologically to be biocompatible. Cell culture permits a fast evaluation of the biological performance of the biomaterials. Thus it is possible to search quickly for the best samples used in the experimental animals. First, we evaluated in vitro the rat fibroblast morphology at the surface of the TLM prosthesis, and then we investigated in vivo tissue colonization after the implantation of the TLM prosthesis in dogs.

From the Departments of Otorhinolaryngology^a and Experimental Surgery,^b Tangdu Hospital, Fourth Military Medical University, Xi'an, China.

Disclosures: Authors have nothing to disclose with regard to commercial support.

Received for publication March 25, 2010; revisions received Aug 25, 2010; accepted for publication Sept 10, 2010; available ahead of print Dec 6, 2010.

Address for reprints: Shiying Cheng, MD, Department of Otorhinolaryngology, Tangdu Hospital, Fourth Military Medical University, Xin Si Road 1, Xi'an, PR China (E-mail: chengshiying1@tom.com).

0022-5223/\$36.00

Copyright © 2011 by The American Association for Thoracic Surgery

doi:10.1016/j.jtcvs.2010.09.042

Abbreviation and Acronym

TLM = metastable β -type titanium

MATERIALS AND METHODS

Prosthesis

The prostheses, in conformity with GB/T16886 standards, were manufactured in collaboration with the Northwest Institute for Nonferrous Metal Research. TLM alloy (consisting of Ti-3, Zr-2, Sn-3, Mo-15, Nb) was used for surgical implants. It was processed first by cutting a TLM alloy plate and drilling tiny holes with a laser (SPI Lasers UK, Southampton, United Kingdom). The diameter of the pores was about 70 to 90 μ m, resulting in a porosity of approximately 30% to 35%. Then the porous TLM alloy plate was placed in a mold for a high-temperature treatment to determine the shape of the prosthesis without vocal fold involvement. Prosthesis sizes were adjusted to the mean diameter of dog cricoid cartilage and trachea, and a rough treatment was given to the surface of the prosthesis. Finally, the prosthesis was polished electrochemically. Prostheses are in a cylindrical C shape and were 0.5 mm thick, 20 to 22 mm in inner diameter, and 20 mm in length. A slot of 3 mm was incised into the tubes, and 7 holes of 1 mm in diameter were pierced at each extremity to fix the prosthesis to the trachea (Figure 1). The pieces obtained were tested for mechanical shock resistance. Before implantation, all prostheses were processed with ultrasonic cleaning and high-pressure sterilization.

Scanning Electron Microscopy

Qualitative information on the morphology of the cells at the surface of the prosthesis was obtained by using scanning electron microscopy. Rat fibroblasts (rat NIH 3T3) were cultured in Dulbecco's modified Eagle's medium containing 15% calf serum, 100 μ g/mL penicillin, and 50 μ g/mL streptomycin in an atmosphere of 5% CO₂ and 95% air at 37°C. After reaching confluence, the cells were collected by means of trypsin digestion and plated at a density of 1×10^5 cells/mL on porous TLM samples placed in 24-well plastic plates. After 4 hours of culture, cells attached to the substrate were washed in phosphate-buffered saline at 37°C, with 2 washes of 2 minutes each. Fibroblasts were fixed for 1 hour in 2.5% glutaraldehyde in phosphate-buffered saline at room temperature. The samples were dehydrated in an ascending ethanol series (50%, 70%, and 90% and 3 times at 100% for 20 minutes each). Specimens were mounted on sample holders with silver print, sputter coated with a gold-palladium alloy in an Hummer JR unit (Siemens, Karlsruhe, Germany), and visualized by means of scanning electron microscopy with a Hitachi S-3400N (Hitachi, Japan, Techcomp, Beijing, China) operating at 5 kV. At least 2 substrates were observed.

Animal Experiments

Ten mongrel dogs weighing approximately 10 to 12 kg were used for the experiment. The operative procedure and the care of the dogs were performed in accordance with the regulations of the Experimental Animal Centre, Fourth Military Medical University. The dogs were acclimatized for 1 week before the operation and monitored for general appearance, activity, excretion, and weight. All experiments were performed after achievement of general anesthesia in a modern operating room. The experimental animals were anesthetized with an intravenous injection of sodium thiopental (10 mg/kg). Anesthesia was maintained with inhaled 1% to 2% isoflurane and 100% oxygen. An antibiotic (cefamandol, 15 mg/kg) was administered intravenously before the incision and during the procedure at the same dose. After anesthesia, the experimental animals were laid on the surgical desk and depilated on the anterior cervical area of the skin (n = 10). Iodine (Shanghai Linkwell Tech Group, Shanghai, China) was used for local sterilization. A sterilized surgical drape was laid

FIGURE 1. Final appearance of the TLM prosthesis with holes at the extremities of each tube (a) and a longitudinal slot (b).

down. Through a vertical midcervical incision of 5 to 6 cm, the larynx and trachea were exposed. The cricothyroid muscles were dissected from the cricoid cartilage and retracted superolaterally. The anterior cricoid cartilage (covering the cricothyroid membrane) with part of the posterior cricoid cartilage plate and the first tracheal ring were simultaneously resected with cold instruments to form an artificial subglottic defect. A thin band of subglottic membrane was conserved to prevent retraction of the larynx and tracheal extremities. The porous prosthesis was anastomosed to the lower edge of the thyroid cartilage on the oral side and to the second tracheal ring on the caudal side with 3-0 absorbable sutures (Figure 2). Finally, the subhyoid muscles were replaced as cover and sutured together on the median line. The operation was finished without an additional airway stent.

After the operation, the animals were caged in a controlled environment with 12-hour on/off cycles. Food and water were provided ad libitum. An antibiotic (cefamandol, 15 mg/kg) was administered intravenously after surgical intervention for 3 days.

Endoscopic Examination

Endoscopic examination was undertaken periodically for each animal after achievement of general anesthesia. The luminal surfaces of the cricoid and trachea were examined with a videoendoscopic system to evaluate the incorporation of each implanted prosthesis with its host tissue and to check for any complications, such as exposure of the porous TLM plate or luminal stenosis. The location and percentage of subglottic stenosis were determined. On the basis of the degree of stenosis found at endoscopy, the animals were assigned one of 3 grades: grade I, approximately 30% to 50%; grade II, approximately 51% to 70%; and grade III, approximately 71% to 100%. Assignment of grades was based on endoscopic findings because this is currently the gold standard for evaluating subglottic stenosis. The videoendoscopic system consists of a video bronchoscope (ENF type V2; Olympus Co Ltd, Tokyo, Japan), a video processor (OTV-ST, Olympus Co Ltd), and a light source (CLH-250, Olympus Co Ltd).

Histologic Examinations

After an observation period ranging from 3 to 8 months, the dogs were killed with an intravenous injection of potassium chloride and propofol. The prosthesis was resected en bloc with both the distal and proximal sites of the native cricotracheal area. After macroscopic examination, the tissues on the TLM alloy were then taken, and part of them were prepared for histopathological examination with hematoxylin and eosin staining and scanning electron microscopic examination.

FIGURE 2. A, The larynx and trachea were exposed. B, The TLM prosthesis was anastomosed to the lower edge of the thyroid cartilage and to the second tracheal ring.

RESULTS

Microscopic Aspect of the Porous TLM Plate and Early Cellular Attachment

Microscopic features of the porous TLM plate were observed *in vitro* by using scanning electron microscopy (Figure 3). Fibroblasts were attached after 4 hours of culture on the porous TLM plate surface. The porous TLM plate presented a quasiuniform image in the diameter of the pores and irregularities on some areas of the surface (Figure 3, A). Cells appeared to have a normal cellular shape with lamellipodia and pseudopodia (Figure 3, B). The porous TLM plate therefore seemed sufficient to sustain cell adhesion and spreading of fibroblasts.

Clinical Results

One dog died of an accident with anesthesia at 1 week. One dog died of pneumonia about 1 month after the operation. The remaining 8 dogs showed good tolerance to the experimental procedure. Eight dogs were killed between the third and eighth months after the operation as follows: 1 dog on the fourth month, 2 on the fifth month, 1 on the sixth month, 2 on the seventh month, and 2 on the eighth month (Table 1).

Endoscopic Examination

The endoscopic examination showed that the cavity of the reconstructed subglottis was free of stenosis. Epithelialization of the luminal surface was observed in the remaining 8 dogs. Granulation (at the proximal or distal anastomotic site of the prosthesis, with the narrowest cavity of the crico-tracheal reconstruction between 8% and 26%) was observed in 4 cases (Table 1; dogs 2, 4, 8, and 9), the porous TLM alloy plate (at the upper anastomotic site of the anterior prosthesis, with an area of about 2×3 mm) was exposed in 1 case (Table 1, dog 4), and anastomotic dehiscence was not observed. However, these dogs were asymptomatic, and they remained in good condition.

Endoscopic findings of a representative dog in the study are shown in Figure 4. A preoperative endoscopic photograph shows the smooth surface of the subglottis (Figure 4, A). Just 1 week after the operation, periprosthetic colonization by connective tissue is already shown in the subglottis, with the benefit of maintaining the air tightness of the prosthesis (Figure 4, B). After 1½ months of implantation, the prosthesis was almost entirely incorporated into the neighboring larynx and tracheal tissue. The anastomotic area was epithelialized with slight swelling (Figure 4, C). After

FIGURE 3. Scanning electron micrograph showing morphologies of rat NIH 3T3 cells cultured for 4 hours on the surface of the TLM prosthesis. A, Holes and cells on the surface of the prosthesis. (Original magnification, $200\times$.) B, Rat NIH 3T3 cells exhibit normal cellular shape with lamellipodia/pseudopodia. (Original magnification, $2000\times$.)

TABLE 1. Results of cricotracheal reconstruction with a prosthesis

Case no.	Implantation time (mo)	Stenosis	Epithelialization	Granulation	Exposure of plate	Incorporation to host	Cause of death
1	8	—	100%	0%	—	+	Killed
2	6	—	92%	8%	—	+	Killed
3	1	—	<40%	0%	—	+	Pneumonia
4	5	—	74%	26%	+	+	Killed
5	0.25	—	0%	0%	—	+	Anesthesia accident
6	8	—	100%	0%	—	+	Killed
7	5	—	100%	0%	—	+	Killed
8	7	—	92%	8%	—	+	Killed
9	4	—	87%	13%	—	+	Killed
10	7	—	100%	0%	—	+	Killed

5 months, the implanted prosthesis was completely incorporated with the host tissue, and the reconstructed area was smoothly epithelialized (Figure 4, D).

Macroscopic Examination

An outer view of the exposed larynx and trachea is shown in Figure 5, A. The TLM plate was completely incorporated into the host tissue. An inner view of the transverse larynx and trachea is shown in Figure 5, B. The luminal surface was almost entirely covered with epithelium without ulceration.

Histologic Examinations

The histologic results of the prosthesis section of the 8 dogs implanted after a period from the third to the eighth months were similar. No abnormal structures were observed at the position of the reconstructed larynx and trachea, and no inflammatory reaction was detected at the area of contact with the biomaterial. The neoepithelium of the reconstructed area extended over the connective tissue layer from both anastomotic sites. Although respiratory epithelium was observed near the anastomoses, only squamous

FIGURE 4. Endoscopic findings before the operation (upper left) and 1 week (upper right), 1½ months (lower left), and 5 months (lower right) after implantation.

FIGURE 5. Macroscopic findings: *left*, outer view of exposed larynx and trachea; *right*, inner view of transverse larynx and trachea. *Trac*, Second ring of the trachea; *R*, reconstructed tissues; *Thy*, thyroid cartilage; *VF*, vocal fold.

epithelium was observed at the midposition of the prosthesis (Figure 6). Scanning electron microscopic examination showed that the cilia on the luminal surface near the anastomoses had no significant differences with those of normal laryngotracheal epithelium.

DISCUSSION

During the past 50 years, a variety of attempts have been made to deal with the challenging problem of the treatment of laryngotracheal stenosis. Schweinfurth¹³ has performed endoscopic treatment successfully on 20 patients with cartilage disease; however, he found the successful treatment was limited when cricoid cartilage was involved. Thus the patients with severe grades of stenosis require an open procedure for expanding the laryngotracheal lumen. Liberman and Mathisen⁹ reported a tailored cricoplasty technique that was applied for a reconstruction of subglottic stenosis in 18 patients, and the results were very good. After the resection of the stenotic lesions, the resulting defect is reconstructed, most commonly through the use of autologous cartilage grafts.^{7,14} Auricular cartilage grafts have been used for tracheal and subglottic reconstruction since the 1950s.¹⁵ Cotton and Evans¹⁶ first reported an anterior cricoid cartilage split with or without an inserted costal cartilage or anterior and posterior cricoid cartilage split with an implanted costal cartilage, and a good result was achieved for the treatment of laryngotracheal stenosis in children. Silva and colleagues¹⁷ reported a success rate of 84% with the auricular cartilage graft. However, the main disadvantage with the autologous cartilage graft was extra incision and surgical trauma at other parts of the body and possible ischemic necrosis of free cartilage. Some cases need to add an intraluminal silicone T-tube to provide support and to maintain airway patency after implantation of autologous tissue. If the T-tube is too rough, the top end is not smooth, or the location is too close to the glottis, the friction could damage the mucous membrane, resulting in the formation of

granulation tissue, which would lead to restenosis and obstruction. In addition, allogeneic materials carry risks of immune rejection, disease transmission, and resorption, whereas synthetic materials are associated with risks of infection, extrusion, and foreign body reactions. Thus far, reconstruction of laryngotracheal defects has been rarely successful mainly because of inflammation and subsequent prosthesis rejection caused by the biomaterial itself.

The term TLM alloy refers to the newly developed metastable β -type Ti, Zr, Sn, Mo, Nb titanium alloys for biomedical application, which seem to be ideal alternative materials for repair of a variety of tissues because of their traits of moderate strength, toughness matching, low elastic modulus, corrosion resistance, easy process, and low cost.¹⁸

The principal concern of biological material application is its safety. In our study we have further tested the biocompatibility of TLM alloy and successfully validated that it is

FIGURE 6. Histological analysis of a colonized section of the cricotracheal prosthesis. *a*, The luminal surface was covered with ciliated columnar epithelium near the anastomoses. *b*, The squamous epithelium was only observed near the midposition. *c*, Connective tissue was in contact with TLM alloy. *L*, Cricotracheal lumen. (Original magnification, 200 $\times$.)

nontoxic and nonirritative as well. We also found that porous TLM alloy obviously could promote the growth of cricotracheal mucous membrane. The endoscopic examination found that the periprosthetic colonization by connective tissue is already present in 1 week, and it prevents the prosthesis from air and bronchial secretion leakage. The experiment showed that material of 0.5 mm in thickness can play well against mechanical forces for supporting the larynx, with laser processing available. Because of its mechanical qualities, an additional intraluminal stent is not required to prevent respiratory collapse after implantation.

The goal of treatment of patients with subglottic stenosis is to provide normal physiologic breathing without the need for a tracheotomy or any further care. The surgical management of subglottic stenosis has evolved over the years. Most of these patients depend on tracheotomies after their operations.¹⁹ Long-term tracheostomies are frequently colonized and infected by bacteria and microorganisms, and thus they might increase the intraluminal contamination despite adequate preoperative care and antibiotics. In our study we used porous TLM alloy to repair the laryngotracheal defect without a postoperative tracheotomy. The microporosity of the material allows vascular and lymphangial permeation, which vascularizes the epithelial colonization.²⁰ The minimal diameter of the pores needed to ensure vascular support is under discussion,²¹ greater microporosity is not aerostatic, and smaller or no microporosity, as in silicone tubes, does not allow surrounding tissue invasion or allow fibroblasts to pass through the wall. However, the study by Scherer and associates²² suggested that the diameter was from 40 to 60 μm . The porosities of the prostheses were kept between 70 and 90 μm in our experiment, and the results were excellent.

Our histologic studies showed few lymphocytes or macrophages present in the tissue surrounding the prosthesis, and the number of inflammatory cells diminished progressively over the period of implantation. The color and luster of the neoeepithelium are similar to those of the native laryngotracheal epithelium, and the cilia on the surface of the mucous membrane were covered well near the anastomoses, but only squamous epithelium was observed at the midposition of the prosthesis. We think that it relates to time of the observation after implantation. Schultz and coworkers²⁰ reported a low or nil endoprosthetic colonization by pseudostratified ciliated columnar epithelium resulting from an inflammatory stenosis of the anastomoses, and their implanted porous titanium prostheses were of 50 mm in length. In contrast, the length of our prosthesis was only 20 mm, and the pseudostratified ciliated columnar epithelium near the anastomoses was higher and larger than that in the study by Schultz and coworkers, which indicates that the length of the implant is obviously one of the very important relevant factors limiting remucosalization of the implanted lumen. In this study we observed a colonization

of fibrous tissue and fibroblasts all around the prosthesis and within the hole of the TLM plate, and these tissues were well vascularized, which would provide a good condition for epithelialization of the prosthetic lumen.

An ideal implanted tracheal prosthesis should keep normal anatomy and physiological status of the implanted lumen for life.²³ In this study the remaining 8 dogs entirely resumed activity after cricotracheal reconstruction and had good cricotracheal anatomy, and no dog had anastomotic or central stenosis (Figures 4 and 5).

At present, significant advances have been made in tissue engineering for laryngotracheal reconstruction. Heatley and colleagues⁶ described that the epithelium would complete cover the auricular cartilage graft in 2 weeks in an animal laryngotracheal reconstruction model. Omori and associates¹⁴ reported a Marlex mesh tube covered by collagen sponge that was applied for laryngeal reconstruction in canines, and a favorable result was obtained. For engineering bone and cartilage, we believe that marrow stromal cells hold the greatest therapeutic potential. The advantage of using stromal stem cells is that obtaining them is less invasive than removing a sample of autologous cartilage. What is advantageous about autologous cartilage is that not only is it very similar to laryngotracheal cartilage, but it is also a source for epithelial cells. However, these cultures often lead to the loss of cilia function and or dedifferentiation of ciliated cells themselves, and furthermore, these reconstructive procedures have required relatively complicated techniques. Simpler and more reliable reconstructive procedure would be the optimal choice.

In this report we performed both in vitro and in vivo experimental tests of the biomaterial to assess its capacity to integrate into biological tissues. We also proved a new technique to reconstruct the larynx and trachea with a prosthesis made of porous TLM alloy. The preliminary data from the current study show confluent regeneration of the epithelium over the TLM alloy and good incorporation of the prosthesis pore into the host tissue 1 week after implantation. However, our study has limitations. Because a thick fibrotic stenosis of the cricoid cartilage and the adjacent tracheal rings is frequently caused by endotracheal intubation,^{1,24} anterior wall opening and insertion of a graft might be required for the repair. With subsequent trauma or damage involved, it is very difficult for physicians to widen the constrained cricotracheal lumen as we expect. Thus additional study with a longer period of observation is necessary with prostheses of longer size. It would be useful, in the future, for clinical repair of long-segment tracheal defects or of partial laryngeal defects.

References

1. Benjamin B. Prolonged intubation injuries of the larynx: endoscopic diagnosis, classification, and treatment. *Ann Otol Rhinol Laryngol Suppl.* 1993;160:1-15.
2. Mitchell SA. The mechanism of tracheal stenosis. *Ear Nose Throat J.* 1979;58:28-33.

3. Shapshay SM, Beamis JF, Hybels RL, Bohigian RK. Endoscopic treatment of subglottic and tracheal stenosis by radial laser incision and dilation. *Ann Otol Rhinol Laryngol*. 1987;96:661-4.
4. Hartley EJ, Cotton RT. Paediatric airway stenosis: laryngotracheal reconstruction or cricotracheal resection? *Clin Otolaryngol*. 2000;25:342-9.
5. Pearson FG, Cooper JD, Nelems JM, Van Nostrand AW. Primary tracheal anastomosis after resection of the cricoid cartilage with preservation of recurrent laryngeal nerves. *J Thorac Cardiovasc Surg*. 1975;70:806-16.
6. Heatley DG, Clary RA, Garner FT, Lusk RP. Auricular cartilage versus costal cartilage as a grafting material in experimental laryngotracheal reconstruction. *Laryngoscope*. 1995;105:983-7.
7. Terra RM, Minamoto H, Carneiro F, Pego-Fernandes PM, Jatene FB. Laryngeal split and rib cartilage interpositional grafting: treatment option for glottic/subglottic stenosis in adults. *J Thorac Cardiovasc Surg*. 2009;137:818-23.
8. Grillo HC. Primary reconstruction of airway after resection of subglottic laryngeal and upper tracheal stenosis. *Ann Thorac Surg*. 1982;33:3-18.
9. Liberman M, Mathisen DJ. Tailored cricoplasty: an improved modification for reconstruction in subglottic tracheal stenosis. *J Thorac Cardiovasc Surg*. 2009;137:573-9.
10. Rack HJ, Qazi JI. Titanium alloys for biomedical applications. *Mater Sci Eng C*. 2006;26:1269-77.
11. Gaafar AA, El-Daly AA, Gaafar HA. Laryngotracheal augmentation using titanium mesh. *J Laryngol Otol*. 2008;122:391-6.
12. Zhentao Y, Yufeng Z, Lian Z, Benli W, Jinlong N, Fuqing H, et al. Shape memory effect and superelastic property of a novel Ti-3Zr-2Sn-3Mo-15Nb alloy. *Rare Metal Materials and Engineering*. 2008;37:1-5.
13. Schweinfurth J. Endoscopic treatment of severe tracheal stenosis. *Ann Otol Rhinol Laryngol*. 2006;115:30-4.
14. Omori K, Nakamura T, Kanemaru S, Kojima H, Magrufov A, Hiratsuka Y, et al. Cricoid regeneration using in situ tissue engineering in canine larynx for the treatment of subglottic stenosis. *Ann Otol Rhinol Laryngol*. 2004;113:623-7.
15. Caputo V, Consiglio V. The use of patients own auricular cartilage to repair deficiency of the tracheal wall. *J Thorac Cardiovasc Surg*. 1961;41:594-6.
16. Cotton RT, Evans JN. Laryngotracheal reconstruction in children five-year follow-up. *Ann Otol Rhinol Laryngol*. 1981;90:516-20.
17. Silva AB, Lusk RP, Muntz HR. Update on the use of auricular cartilage in laryngotracheal reconstruction. *Ann Otol Rhinol Laryngol*. 2000;109:343-7.
18. Zhentao Y, Lian Z, Lijuan L, Maohng F, Tanyan F. Investigation on mechanical compatibility matching for biomedical titanium alloys. *Key Eng Mater*. 2005;288-299:595-8.
19. Younis RT, Lazar RH, Astor F. Posterior cartilage graft in single-stage laryngotracheal reconstruction. *Otolaryngol Head Neck Surg*. 2003;129:168-75.
20. Schultz P, Vautier D, Charpiot A, Lavalle P, Debry C. Development of tracheal prostheses made of porous titanium: a study on sheep. *Eur Arch Otorhinolaryngol*. 2007;264:433-8.
21. Nelson RJ, Goldberg L, White RA, Shors E, Hirose FM. Neovascularity of a tracheal prosthesis/tissue complex. *J Thorac Cardiovasc Surg*. 1983;86:800-8.
22. Scherer MA, Ascherl R, Geissdorfer K, Mang W, Blümel G, Lichti H, et al. Experimental biosynthetic reconstruction of the trachea. *Arch Otorhinolaryngol*. 1986;243:215-23.
23. Jhngook K, Soo WS, Ji YS, Jin HK, Yong SC, Hojoong K. Replacement of a tracheal defect with a tissue-engineered prosthesis: Early results from animal experiments. *J Thorac Cardiovasc Surg*. 2004;128:124-9.
24. Henderson JH, Welter JF, Mansour JM, Niyibizi C, Caplan AI, Dennis JE. Cartilage tissue engineering for laryngotracheal reconstruction: comparison of chondrocytes from three anatomic locations in the rabbit. *Tissue Eng*. 2007;13:843-53.