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Esophageal reconstruction: Combined application of muscle tissue flap and inner chitosan tube stent in rabbits

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Abstract.
BACKGROUND: Esophageal reconstruction is the key issue in esophageal surgery. However, currently there is no satisfied technique to repair esophagus after surgery.
OBJECTIVE: To combine an inner chitosan stent and a muscle tissue with vascular pedicle to repair the esophageal defect in cervical segment.
METHODS: Esophageal defect was repaired using the combination of a muscle tissue flap and a chitosan tube stent in experimental group while only muscle tissue flap was utilized in controls for comparison. One animal in each group was sacrificed at week 3, 6 and 9 after operation respectively to exam the healing status. Barium X-ray was used to evaluate the esophageal status in 12 weeks.
RESULTS: Histology showed the inflammatory response in 3 weeks after surgery, the chitosan stent was partially absorbed in 6 weeks, and there was no obvious fibrotic proliferation in experimental group; while the fibrotic proliferation and esophageal stenosis were obvious in controls, the chitosan stent was completely absorbed in 9 weeks, and squamous epidermis cells were observed. Twelve weeks later, the barium swallow went smoothly through the esophagus with noticeable peristalsis in the experimental group; esophageal stenosis without peristalsis was observed in controls.
CONCLUSION: The combination of chitosan stent and muscle tissue flap is feasible to reconstruct a partial defect in esophagus.

Keywords: Chitosan, stent, muscle flap, esophagus defect, esophagus reconstruction

1. Introduction

Partial esophageal resection is a kind of approach to cure esophageal cancer and benign esophageal stenosis. The esophagus needs to be reconstructed after the surgery in order to complete the integrity of the esophagus. Esophageal reconstruction technology is of great significance to esophageal surgery. Esophageal substitution is a hot research topic [1,2], and has been studied for about seventy years but with less progress. There are two kinds of substitutions in esophageal surgery, i.e., self tissue and artificial substitutes. The widely used method for clinical esophageal reconstruction is to use the hollow self organization substitutes, such as the stomach, colon and jejunumetc [3]. Other self-tissue includes

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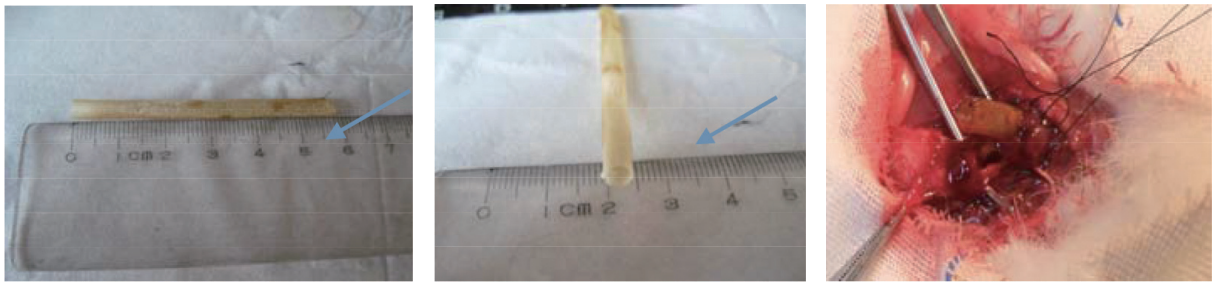


A B C

Fig. 1. Chitosan tubular stent with the diameter of 4 mm, the pipe wall thickness of 0.5 mm, and the length of 60 mm. (A/B); The tubular stent was then cut into 6 segments, each was 10 mm long, which was used to reconstruct the esophagus defect (C).

muscle and skin etc. [4,5]. Due to the disruption of the normal function of the digestive tract, some post-operative adverse effects will be inevitable, and a shift of the organ causes damage to the tissue. To some patients or individuals with gastrointestinal abnormalities, this kind of approach for esophageal reconstruction is restricted. Clinical researchers have been looking for the artificial esophagus, just as artificial blood vessels, which may replace the part of esophagus defect and maintain the esophageal integrity. Artificial esophagus may be made of various materials, including plastic, metal, silicone, polyethylene, biological tissue and biological composite materials. The approaches for applying these substitutions are diverse, including autologous implantation, such as autograft, allograft and implantation of biological materials. Tissue engineering of esophageal reconstruction is still in the stage of the laboratory research. Researchers have tried several artificial esophagi in recent years. However, the clinical outcome is far from satisfaction.

Our group used the lung tissue flap to repair the esophageal defects in thoracic location in situ in experimental animals. Based on the previous experimental success [6], this paper designed and created the absorbable chitosan stent, combined with surrounding muscle tissue flap, to reconstruct esophageal defect in situ in cervical segment. The feasibility of this technique was evaluated.

2. Methodology

2.1. Chitosan tube stent preparation

Eight percent of chitosan acetic acid hydrosol was prepared by the dissolution of 16 g chitosan powder into 200 ml distill water with 2 ml acetic acid. One day later, a 80 mm long hard plastic tube with a diameter of 5 mm was briefly immersed into the 8% chitosan acetic acid hydrosol followed by the incubation in l mol/L sodium hydroxide solutions for 3 minutes. When the hydrosol turned into jell and became semi-dry, the inner tube was gently removed and replaced by another slightly thinner plastic tube. The dried chitosan tubular stent was of 4 mm in diameter, 0.5 mm in wall thickness, and 60 mm in length. (1A, B). The tubular stent was then cut into 10 mm long and sterilized into ethylene oxide for 4 hours in aseptic packaging standby, in preparation for the surgery of esophagus reconstruction (1C).

2.2. Experimental animals and materials

Twenty-three Japanese big ear white rabbits, each weighing 2–3 kg, were purchased from Shengjing Hospital Animal Center, China Medical University (Shenyang, China). They were divided into two

groups: control group ($n = 8$), experimental group ($n = 15$). The chitosan tubes, 10 mm in length and 4 mm in inner diameter, were made by the authors. Antiseptic ethyleneoxide was from Yongding Disinfectant Equipment Factory (Beijing, China), 10% chloralhydrate from Yulong Algae Co. (Qingdao, China) and 2% lidocaine from North China Pharmaceutical Ltd. (Shijiazhuang, China). Other materials including stomach tube, needle and medical adhesive plaster were purchased from North China Pharmaceutical Ltd. (Shijiazhuang, China). Digital camera (Sony, DSC-T10, Tokyo, Japan), and optical microscope (Olympus CH-20, Tokyo, Japan) were used.

2.3. Surgery preparation

Experimental animals were fasted for 12 hours before operation. 10% chloralhydrate (5 mL/kg) was injected peritoneally. 1% lidocaine (1 mg/kg) was used as local anesthetic and injected into cervical incision wound to reduce the pain. The stomach tube was placed. The anaesthetized rabbits in supine position were fixed on the operation table, and the front tooth and lower jaw were pulled with the bandage by the assistant in the flank. The head side was the operator. The fur was cleaned and the skin was sterilized,

2.4. Surgery

The left side skin in the cervical segment was cut and the muscles were exposed in the neck. The esophagus was found under the trachea. The esophagus was supported by stomach tube inserted inside, and it was easy to search the center-section for its dissociation, and slung with sterile gauze strip. Esophageal central segment defect was 3–4 mm long and 1/2–2/3 in circumferences. In the experimental group, the tubular chitosan stent in the esophagus cutting was placed and sutured with thread. Muscle tissue petal nearby wrapped the esophagus in the cutting to form an encystation, and continuously sutured oesophagus cutting edge with a 3-0 silk thread. Then, the stomach tube was withdrawn to release the stress. The procedure of controls was the same but no insertion of chitosan tube. No hemorrhage and other injury were found. Normal saline was injected into the stomach tube to confirm that there was no leakage in muscular flap repair area. The muscle was sewn up and the skin was sutured to finish the surgery. The rabbits received anti-infection treatment (gentalmycin 40,000 unit per day for 5 days), no diet for 5 days but 100 ml glucose-saline intravenous administration, liquid diet for 2 days, and normal diet afterwards.

2.5. Clinical observation, sample collections and histology

The animals were monitored after operation. The survival rate, diet, body weight and complications were recorded. One rabbit in each group was killed at week 3, 6 and 9 after surgery, respectively. The esophageal damage, the healing status and the chitosan stent were observed; the muscle flap was fixed in 10% formalin, stained with hematoxylin and eosin (HE) and the histology was evaluated under the optical microscopy. Twelve weeks later, the status of the esophagus was observed by barium meal in survived rabbits.

3. Results

3.1. Outcomes of the animals

One animal in experimental group died of infection on day 7 after surgery, one in control died of severe esophageal stenosis and malnutrition 2 weeks after operation. Fourteen rabbits from experimental group

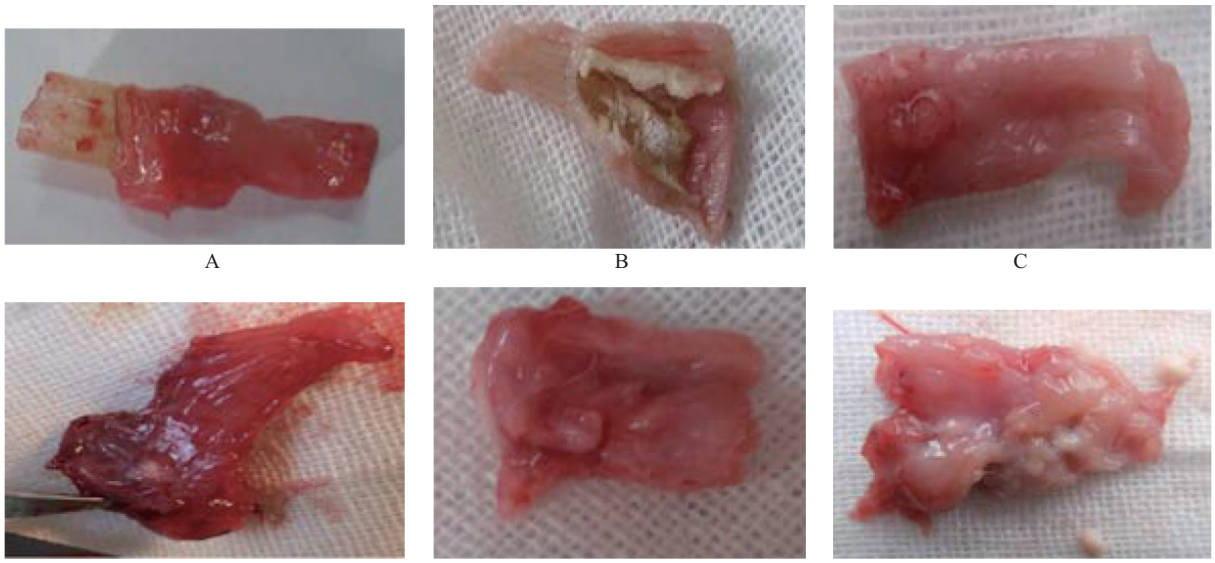


Fig. 2. Muscle tissue flap change in experiment group at 3 (A), 6 (B) and 9 (C) weeks after operation; the corresponding changes in control group were shown as D, E, F.

and 7 from controls were survived. All of the rabbits had normal diet within 2 weeks after surgery, the body weight decreased within 0.5 kg in experimental group; the diet decreased significantly in control animals at week 2 after operation and the body weight was reduced by 0.5–1 kg. There was no significant decrease in food intake 2 weeks later after surgery in experimental group and the body weight had no significant change.

3.2. Gross morphology

Three weeks later after surgery, the muscle tissue flap had a good blood supply and was healed at the esophageal defect area. Opening the esophageal wall, it can be seen that the inner chitosan stent was intact without obvious migration. There was membrane-like tissue inside the esophageal surface of the defect site in animals of both groups. Six weeks later after surgery, the muscle tissue flap completely covered the esophageal defect area. There was un-absorbed threat and no tissue edema. Once opening the esophageal wall, the inner surface of the esophageal was observed to be smooth and tube-like frame. The esophageal surface had particle feeling when touched, while the control group had integrated esophageal surface with tissue proliferation and edema. Nine weeks later after surgery, the esophageal defect in experimental animals was completely repaired by muscle tissue flap; the surface was healed from both upper and lower esophageal extensions; and the chitosan stent was completely absorbed without obstruction and ulcer. By comparison, though the muscle tissue flap was also completely healed in controls without obvious line between the defect area and the original untouched esophageal surface, the tissue proliferation in defect area was obvious and there was esophageal stenosis in that region (Fig. 2).

3.3. Histology

Three weeks after surgery, the muscle substitutes were intact in both groups accompanied with inflammatory response, such as inflammatory cell infiltration. Six weeks after surgery, the structure of the

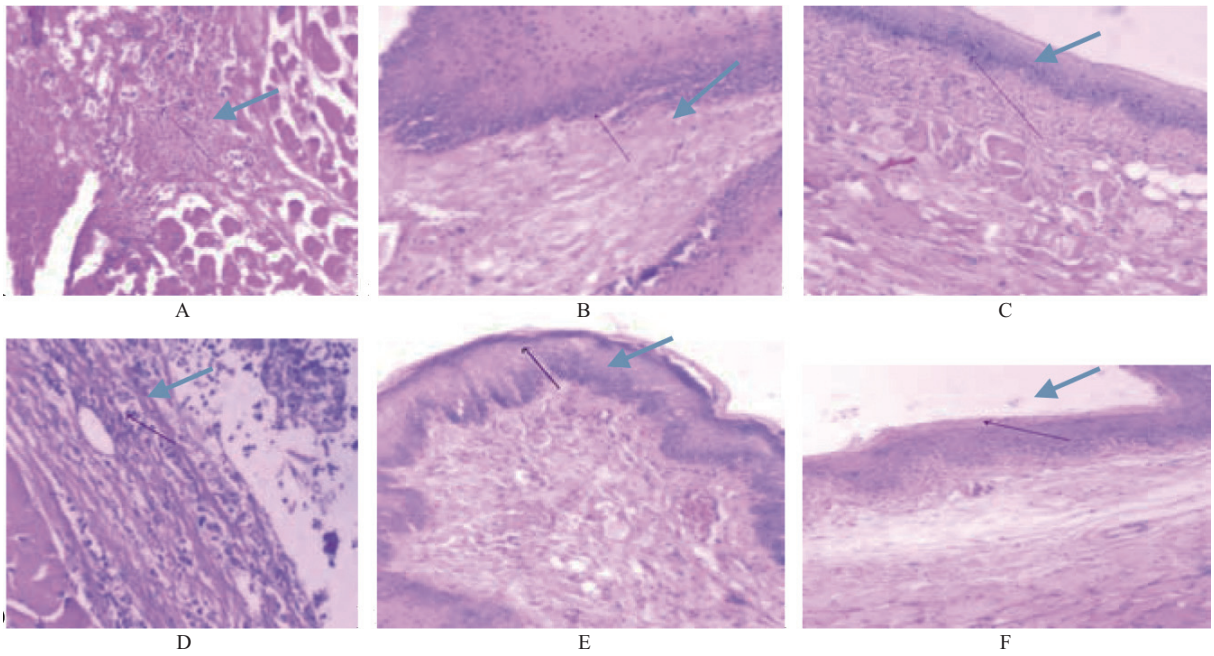


Fig. 3. Muscle tissue flap change under optical microscope in experimental group at 3 (A), 6 (B) and 9 (C) weeks after operation; the corresponding changes in control group were shown as D, E, F. (HE, × 40), respectively. A and D: inflammatory cell infiltration; B: few neutrophil and lymphocyte filtrations, E: fibrotic cell proliferation; C: squamous metaplasia on the surface of muscle tissue flap, F: fibrotic tissue proliferation.

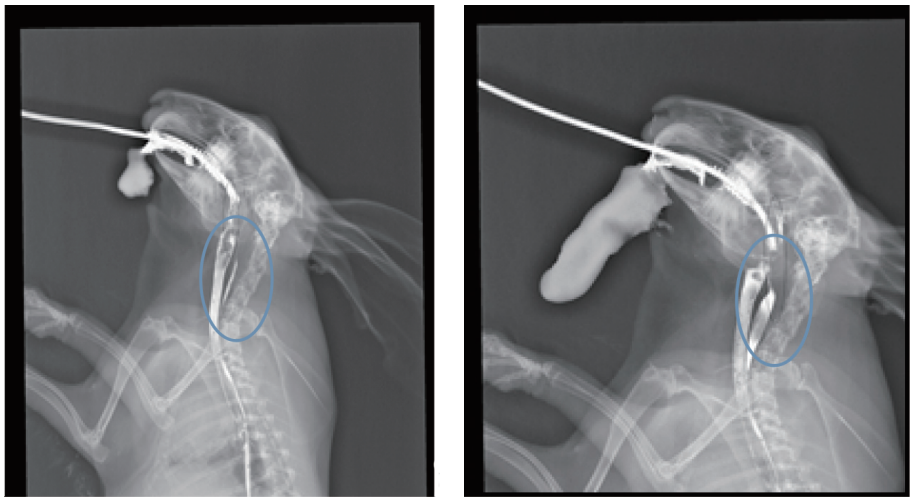
muscle tissue flap substitutes was clear with few neutrophil and lymphocyte filtrations in experimental animals; there was no significant tissue proliferation. In control animals, fibrotic cell proliferation and inflammatory cells were found on the surface of muscle tissue. Nine weeks after surgery, there was squamous metaplasia on the surface of muscle tissue flap in experimental group, the mucous membrane extended from both upper and lower sides of the esophagus, and the chronic inflammatory response under the mucous membrane was significantly alleviated compared with that in 6 weeks. In control group, there were fibrotic tissue proliferation on the surface of muscle tissue, and there were no squamous metaplasia and no mucous creeping (Fig. 3).

3.4. Barium X-ray

Twelve weeks after operation, barium meal showed smooth esophagus in experimental group. There was no obvious stricture, reflux, anastomotic stoma leakage and dilatation. The peristalsis was noticeable. In control group, there was stenosis in repaired region and esophageal dilatation above the stenosis. Peristalsis in the cervical segment of esophagus was not observed (Fig. 4).

4. Discussion

To our knowledge, this is the first study using the combination of muscle tissue flap and chitosan stent to repair the esophageal defect in cervical segment. Histology showed that this combination promoted squamous metaplasia on the surface of muscle tissue flap, the mucous membrane extended from both



A B

Fig. 4. Twelve weeks after surgery, in experimental group (A), barium meal went smoothly through the repaired esophagus, no obvious stricture, reflux, anastomotic stoma leakage and dilatation were observed; and in the control group (B), there was stenosis in repaired region and esophageal dilatation above the stenosis.

upper and lower sides of the esophagus; barium X-ray demonstrated that there was no stenosis and barium reflux, the reconstructed esophagus had noticeable peristalsis. In comparison, esophageal defect repaired by muscle tissue flap only showed more fibrotic proliferation, stenosis, barium reflux and the absence of squamous metaplasia and peristalsis. These data validated the feasibility of the combination of muscle tissue flap and chitosan stent in esophageal reconstruction.

The esophageal reconstruction is essential in esophageal surgery and the selection of esophageal substitutes is a challenging issue for researchers [7,8]. Esophageal stent is indeed essential for the success of the esophageal reconstruction [9]. Le Baleur and his colleagues did 12 esophageal replacement in pigs, five of which used aorta as a substitute without stent, and other seven implanted aorta combined with removable stent made of silicone with polyester mesh. All animals without stent insertion died of anastomotic leakage within 20 days of surgery. Five out of the 7 stented animals survived for 1 month in good clinical condition [10]. Le Baleur's study implied that the stent implantation is a must in esophageal reconstruction. One can imagine that improvements in stent design and materials, currently being undertaken, are essential in esophageal reconstruction. Early studies focused on the synthesis of artificial esophagus, such as metal, plastics, polyester fiber, silicone or combined materials [11]. However, these prosthetic materials cause foreign body reaction and therefore, replacement by artificial materials has not been proven to be clinically useful because of their absence of biocompatibility, which leads to chronic infection, anastomotic leakage, migration, and stenosis [9]. Allograft or autograft using various kinds of tissue, such as pleura, pericardium, skin [12], muscle fascia, jejunum or colon have also been proven to be unsuccessful (1). Recent efforts in the field of tissue engineering have not yet provided reliable results [13].

The ideal material for esophageal stent should be: 1. biocompatible; 2. biodegradable, where the degradation speed should be faster than that of the proliferation of esophageal tissue; 3. resist the reflux of gastric juice from the stomach; 4. should not cause infection and inflammation [14]; 5. nontoxic. Chitosan seems fit all categories above. Chitosan is from chitin which is the only high polymer material with widespread biodegradation [15], and it has good biological compatibility with animal organs and cells without immunogenicity [16].

Our present study showed that chitosan tolerated well to gastric juice, suppressed inflammation response and prevented the scar and adhesion in the damaged section. Chitosan is nontoxic and can be degraded to low molecular oligosaccharide, and it is not accumulated.

After trial and error, our group successfully combined auto-pulmonary tissue flap with chitosan stent to repair thoracic trachea and esophagus defect [6,17]. The present study demonstrated the same result in cervical esophagus defect reconstruction. Instead of using lung tissue flap [6], this paper employed muscle tissue flap with vascular pedicle to form the package in the disrupted place. The advantage of using muscle tissue flap is that muscle tissue is next to the esophagus and ready to use and there is no need to worry about rejection. The surgical technique is simple, as the original physiological esophageal tract is unchanged, avoiding opening thoracic and abdomen The present study showed that after executing the supporting function and preventing the formation of stenosis, chitosan was automatically degraded. We did No toxic affect and rejection response were found. Compared with muscle tissue flap only, chitosan stent significantly alleviated the inflammatory response and inhibited fibrotic tissue proliferation.

In conclusiončňchitosan is completely biocompatible to the surrounding esophageal tissue, has no toxic affect, does not trigger inflammatory and rejection response and inhibits fibrotic tissue proliferation. Therefore, chitosan is an ideal material for the esophageal stent in esophageal reconstruction.

Acknowledgement

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References

[1] Shen Q, Shi P, Gao M, Yu X, Liu Y, Luo L, et al. Progress on materials and scaffold fabrications applied to esophageal tissue engineering. Mater Sci Eng C Mater Biol Appl. 2013; 33(4): 1860.
[2] D'Journo XB, Martin J, Ferraro P, Duranceau A. The esophageal remnant after gastric interposition. Dis Esophagus. 2008; 21(5): 377.
[3] Ninomiya I, Okamoto K, Oyama K, Hayashi H, Miyashita T, Tajima H, et al. Feasibility of esophageal reconstruction using a pedicled jejunum with intrathoracic esophagojejunostomy in the upper mediastinum for esophageal cancer. Gen Thorac Cardiovasc Surg. 2014; 62(10): 627.
[4] Miyamoto S, Kayano S, Fujiki M, Sakuraba M. Combined use of the cephalic vein and pectoralis major muscle flap for secondary esophageal reconstruction. Microsurgery. 2014; 34(4): 319.
[5] Chen HC, Kuo YR, Hwang TL, Chen HH, Chang CH, Tang YB. Microvascular prefabricated free skin flaps for esophageal reconstruction in difficult patients. Ann Thorac Surg. 1999; 67(4): 911.
[6] Chen G, Shi WJ. Lung tissue flap repairs esophagus defection with an inner chitosan tube stent. World J Gastroenterol. 2009; 15(12): 1512.
[7] Parekh K, Iannettoni MD. Complications of esophageal resection and reconstruction. Semin Thorac Cardiovasc Surg. 2007; 19(1): 79.
[8] Lerut T, Coosemans W, Decker G, De Leyn P, Nafteux P, van Raemdonck D. Anastomotic complications after esophagectomy. Dig surg. 2002; 19(2): 92.
[9] Gaujoux S, Le Balleur Y, Bruneval P, Larghero J, Lecourt S, Domet T, et al. Esophageal replacement by allogenic aorta in a porcine model. Surgery. 2010; 148(1): 39.
[10] Le Baleur Y, Gaujoux S, Bruneval P, Lambert B, Larghero J, Cattan P, et al. Self-expanding removable plastic stents for the protection of surgical anastomoses after esophageal replacement in a porcine model. Gastrointestendosc. 2010; 72(4): 790.
[11] Freud E, Efrati I, Kidron D, Finally R, Mares AJ. Comparative experimental study of esophageal wall regeneration after prosthetic replacement. J Biomed Mater Res. 1999; 45(2): 84.
[12] Horvath OP, Cseke L, Borbely L, Vereczkei A, Hobor B, Lukacs L. Skin tube esophagus: present indications and late malignization. Dis Esophagus. 2000; 13(3): 251.

[13] Totonelli G, Maghsoudlou P, Fishman JM, Orlando G, Ansari T, Sibbons P, et al. Esophageal tissue engineering: a new approach for esophageal replacement. World J Gastroenterol. 2012; 18(47): 6900.
[14] Kuppan P, Sethuraman S, Krishnan UM. Tissue engineering interventions for esophageal disorders – promises and challenges. Biotechnol Adv. 2012; 30(6): 1481.
[15] Dooley TP, Ellis AL, Belousova M, Petersen D, DeCarlo AA. Dense chitosan surgical membranes produced by a coincident compression-dehydration process. J Biomater Sci Polym Ed. 2013; 24(5): 621.
[16] Shi H, Wang W, Lu D, Li H, Chen L, Lu Y, et al. Cellular biocompatibility and biomechanical properties of N-carboxyethylchitosan/nanohydroxyapatite composites for tissue-engineered trachea. Artif Cells Blood Substit Immobil Biotechnol. 2012; 40(1-2): 120.
[17] Shi WJ, Zhang SN, Yang W, Zhao JG, Zhao Y, Liu J. Clinical application and animal experiment of thoracic tracheal reconstruction by using pulmonary tissue flap. Zhonghuawaikezazhi. 2003; 41(3): 218.