ORIGINAL ARTICLE
FACIAL PLASTIC AND RECONSTRUCTIVE SURGERY
DOI: 10.5152/B-ENT.2023.221153

Application of Xenogenic Acellular Dermal Matrix for Reconstruction of Cervical Tracheal Defects in a Rabbit Model

Ping Li, Shisheng Li, Jinkun Li, Xinming Yang

Department of Otolaryngology-Head and Neck Surgery, The Second Xiangya Hospital, Central South University, Changsha, Hunan, China

Cite this article as: Li P, Li S, Li J, Yang X. Application of xenogenic acellular dermal matrix for reconstruction of cervical tracheal defects in a rabbit model. *B-ENT*. 2023;19(2):74-79.

ABSTRACT

Objective: The aim of this study was to explore the safety of xenogenic acellular dermal matrix for tracheal reconstruction and to explore the process and effectiveness of mucosal repair.

Methods: A total of 22 rabbits were divided into the control group and the experimental group. A 3-ring, 120-degree tracheal patch was resected, and the tracheal wall was reconstructed by xenogenic acellular dermal matrix (experimental group) or cervical fascia (control group). Symptoms of the animals, morphology, and micromorphology of the reconstructed tracheas area were evaluated at 1, 4, 8, 12, 16, and 24 weeks postoperatively.

Results: In the control group, 1 rabbit got subcutaneous emphysema and the rabbit died of airway obstruction. Tracheal stenosis was observed in all the animals. In the experimental group, no animal showed ingrowth of granulation tissue or accumulation of secretion of the trachea. The acellular dermal matrix got rapidly vascularized and epithelized with ciliated cells. Goblet cells could be observed 24 weeks postoperatively.

Conclusion: The tracheas were successfully reconstructed by the acellular dermal matrix, and they are stable with well-functional epithelial lining. This is a simple, safe, and effective surgical method with few complications. As a medical device, this thin membrane would expand the choice of repair materials during tracheal reconstruction.

Keywords: Acellular dermal, animal experimentation, epithelium regenaration, reconstruction, trachea

Introduction

Malignant tumors of the cervical trachea are encountered often during clinical work, and surgery is the preferred treatment. Although many tumors can be surgically cured, reconstruction surgery is difficult because of the complex structure and unique blood supply of the trachea.[1] A healthy trachea has an epithelial lining with a rigid open lumen. In the process of respiration, the cartilage framework must reserve the open lumen under change in pressure. The mucosal epithelium is airtight and keeps the trachea clean. The epithelial lining and anatomical structure of the trachea could be maintained by end-to-end anastomosis. However, the maximum length has been limited by the complex structure and unique blood supply of the trachea, which was determined to be 6 cm. The risk of crack and stenosis increases with increasing defect length.[2] In fact, only few cases require circumferential resection for confined lesions. Window resection is a surgical method that only excises part of the tracheal ring; the advantage of this method is lower invasiveness than circumferential resection and the airway can be certainty secured.[3] An autologous myocutaneous flap graft is always viable and reliable.[4] However, the disadvantages are obvious such as pain, muscle protrusion, and unaesthetic appearance of the donor site. Complications such as infection and hemorrhage are possibly lethal.[5] The use of a tracheal substitute would solve such problems and make reconstruction both safer and easier.[6]

In the review literature, the ideal tracheal substitutes should be airtight and should have minimal collapse, good organizational compatibility, and rapid vascularization for nutrient transportation.[7] Despite many reports and studies on engineering materials, such as 3D-printed scaffold, polyethylene glycol grafted and porous polycaprolactone/pluronic membrane were tried in animal models, there was still no guideline available for tracheal reconstruction.[8-12] Acellular dermal matrix (ADM) is

Corresponding author: Xinming Yang, e-mail: yangxinming@csu.edu.cn
Received: October 8, 2022 **Accepted:** February 12, 2023 **Publication Date:** April 27, 2023
Available online at www.b-ent.be

a homogeneous extracellular matrix that is processed from corpse skin. It promotes cellular repopulation and migration that guides normal tissue regeneration as a natural template, which implies ADM can undergo rapid epithelialization and vascularization. Acellular dermal matrix was initially utilized in burn surgery[13] and has then been widely applied clinically.[14,15] Xenogenic acellular dermal matrix (xeno-ADM) is a medical device that is derived from fetal cattle skin. It is similar to ADM in biological properties and is also widely used in clinic. The source of xeno-ADM is extensive and its lower-price adaptation to clinical needs much better. We have previously reported that application of xeno-ADM for hypopharyngeal mucosa repair in 40 patients; the swallowing is superior to that of traditional method.[16] Xeno-ADM was previously used to reconstruct the trachea defect in 2 patients, the trachea remained its diameter and the reconstructed areas were clean and airtight.[17] However, the safety of xeno-ADM in tracheal reconstruction and the process of mucosal repair need further exploration.

The purpose of this study was to evaluate xeno-ADM further to: (1) avoid granulation tissue formation to maintain airway patency, (2) allow rapid vascularization and epithelization, and (3) restore a functional cilium lining for reconstruction of a stable and functional trachea.

Methods

After the approval of this study by the Animal Ethics Committee of Central South University (ethical approval number: 2019-194), animals were cared for strictly in accordance with institutional guidelines. All procedures were conducted under anesthesia by an intravenous injection (ear vein) of 1% pentobarbital sodium (30 mg/kg) to minimize suffering. This study follows the ARRIVE Guidelines 2.0 as reporting guideline.[18]

Xenogenic Acellular Dermal Matrix

The xeno-ADM (fetal cattle), as a medical device, was provided by Yantai Zhenghai Biologic Technology Ltd Co. The specifications of the xeno-ADM are 1.5 cm × 2 cm; and thickness ranged from 0.30 to 0.39 mm (authorized number: 2011-3460301).

Animal Studies

All surgical processes and management of animals were conducted following the regulations of the Institutional Review Board for animals. The animals used for this study were 22 male New Zealand rabbits, weighing 2.2-2.7 kg and were numbered 1 to 22 randomly. The animals were provided by the Laboratory Animal Center of our hospital. There were 10 rabbits (nos 1-10) in the control group and 12 rabbits (nos 11-22) in the experimental group. Four rabbits (2 rabbits in each group) got sacrificed at each time point (4, 8, 12, 16, and 24 weeks postoperatively, 2 rabbits were sacrificed 1 week postoperatively in the experimental group to evaluate the vascularization of the ADM). The rabbits were acclimated for 5 days before the initiation of the experiments.

Main Points

- Tracheal reconstruction is difficult because of its unique anatomical characteristics. Reconstruction surgery of the trachea with artificial graft needs further exploration.
- Acellular dermal matrix could be used as a thin membrane for mucosal repair and could be used for tracheal reconstruction.
- The reconstructed trachea is stable and maintained its rigid lumen, and the epithelial lining is well functional after being repaired by acellular dermal matrix.

The surgical procedure was as follows (shown in Figure 1). A 3-ring, 120-degree tracheal wall was resected. A trachea defect of this size has been confirmed that would result in serious stenosis of the trachea and subsequent death in rabbits while without repairing.[19] In the control group, the defect was reconstructed by cervical fascia alone. In the experimental group, the defect was reconstructed by ADM with 6-0 absorbable thread and reinforced by cervical fascia. The ADM was immerged in 0.9% sodium chloride solution for 30 minutes before use and the basement membrane surface faced to the lumen. The strap muscles were sutured with 4-0 nylon thread. Then, the rabbits were observed for 2 hours before being returned to their cages, and water and feed were freely available. The animals were given 50 000 IU/kg penicillin as prophylaxis for the following 3 days. Clinical symptoms, with special attention to wheezing, cough, sputum production, and dyspnea, were monitored daily.

Endoscopic Examination

Rabbits were anesthetized to evaluate the repaired trachea. Bronchoscopy was performed by a 2.7-mm 0° rigid endoscope. Images and videos were taken for each rabbit's airway with an IKEDA 9003 endoscopic camera attached to the rigid endoscope.

Histologic and Electron Microscopic Examination

The animals were euthanized, and the repaired tracheal segments were harvested after endoscopic airway examinations. The reconstructed area was divided into 2 averaged parts. For histologic microscopic examination, the tracheas were embedded in paraffin, cut at a thickness of 4 μm, and stained with hematoxylin and eosin. For scanning electron microscope (SEM) examination, the samples were prefixed by immersion in 2% glutaraldehyde in 0.1 M phosphate-buffered solution, and post-fixed for 2 hours in 1% osmic acid dissolved in phosphate-buffered solution. Samples were treated in a graded series of ethanol and *t*-butyl alcohol, and dried in freeze dryer platinum coated using an ion coater and observed under a TM3030 scanning electron microscopy. In transmission electron microscope (TEM) examination, sample was processed as for SEM, then was cut at a thickness of 100 nm and dyed by 3% of uranium acetate and lead nitrate. The sample was observed under a S3400 transmission electron microscopy.

Results

In the control group, 1 of the 10 rabbits (no. 2) got subcutaneous emphysema, and anti-infective treatment was prolonged until the emphysema was absorbed. Unluckily, the rabbit died in the third week after the surgery.

In endoscopic examination, serious tracheal stenosis was observed in all the rabbits in the control group. Sputum scab was retained on the reconstructed area 4 weeks postoperatively (no. 1, shown in Figure 2A).

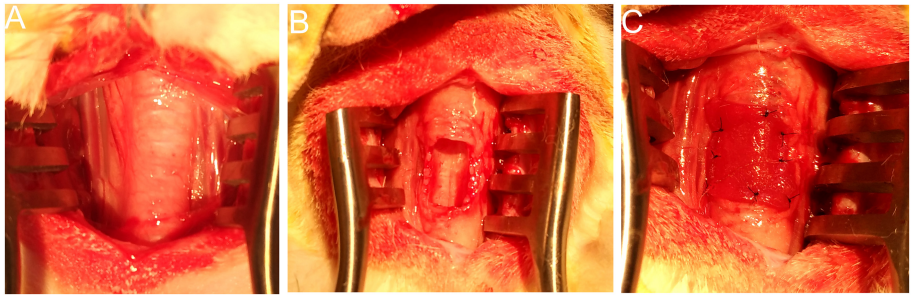

Figure 1. Surgery photos. (A) Strap muscles were separated and the tracheal cartilages were encountered. (B) A 3-ring, 120-degree tracheal wall was resected. (C) The ADM was sutured to the defect.

In the experimental group, none of the rabbits showed an ingrowth of granulation tissue on the inner surface of the ADM, with no accumulation of secretions. Vessels were observed in ADM, and the reconstructed areas were clean and airtight the fourth weekend after reconstruction (shown in Figure 2B). Airway stenosis was minimal, and the trachea remained its lumen (shown in Figure 2C).

In the control group (no. 2), a granulation sized 4 mm × 4 mm and sputum retention were observed in the tracheal lumen (shown in Figure 2D), which may cause the obstruction of the airway and death. In the experimental group, the reconstructed tracheas were clear and smooth. The ADMs completely fused to the tracheas without granulation formation or dislocation. No graft failure was identified.

In the control group, the respiration epithelium was interrupted at the cut edge without any sign of crawling (8 weeks postoperatively, shown in Figure 3A). In the experimental group, the ADM is uncovered at the first weekend postoperatively, while capillaries and lymphocyte infiltration were observed (shown in Figure 3B). Four weeks after surgery, the ADM is partly covered by epithelium, while the central area was still bare (shown in Figure 3C). Respiratory epithelium covered all the ADM 8 weeks postoperatively, and at this moment, the ADM got degraded (shown in Figure 3D).

The epithelium cells were different in shape and size at first, and villus with a few cilia could be observed (shown in Figure 4A). The cilia were more trimmed at the eighth week after the surgery (shown in Figure 4B). At the 24th week after surgery, the reconstructed area has been covered by neatly ciliated epithelium (shown in Figure 4C), comparable to that of a normal trachea. Goblet cells could be observed in the regenerated epithelium on TEM examination (shown in Figure 4D).

Discussion

There are 2 broad issues that clearly need to be considered in trachea reconstruction surgery. First, it is required to maintain the luminal form and epithelium lining; second, the postoperative restenosis should be prevented. Autologous grafts such as pericardium, pleural, and periosteum could use for reconstruction of small defects.[20] For larger tracheal defects, flap with guaranteed blood supply and skeletal support is required to ensure the reconstruction, and the keratinocytes can well act as an airway lining.[21] However, the myocutaneous flap cannot undergo re-epithelialization, and the hair of the flap easily leads to itching and sputum retention.[22] The use of an artificial prosthetic trachea, however, faces the same limitations of poor epithelialization.[23] Trachea maintains the homeostasis by mucociliary clearance of the cilia. Thus, timely re-epithelialization is required to prevent respiratory compromise and

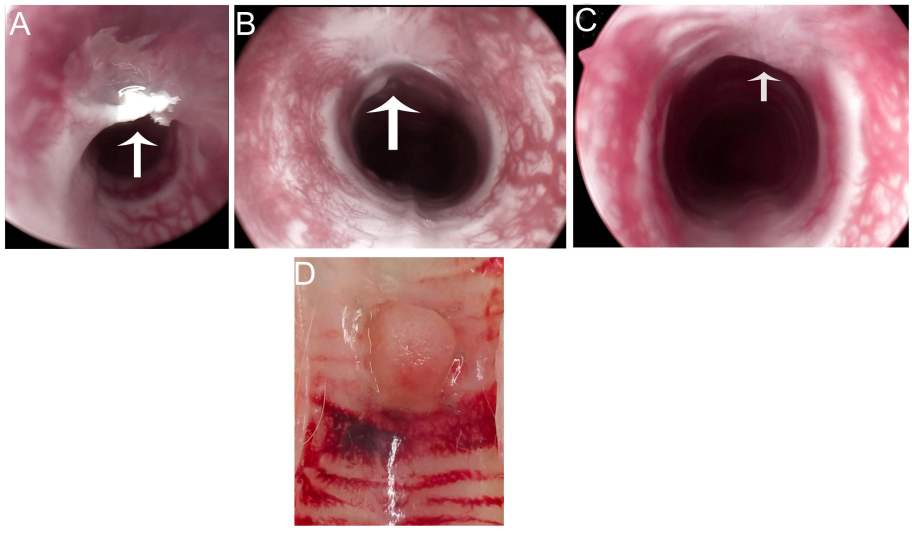

Figure 2. Endoscopic images and gross finding of the reconstructed area. (A) Tracheal stenosis and sputum retention on reconstructed area in the control group. (B-C) Well-maintained airway lumen in the experimental group. (D) The granulation tissue in rabbit no. 2.

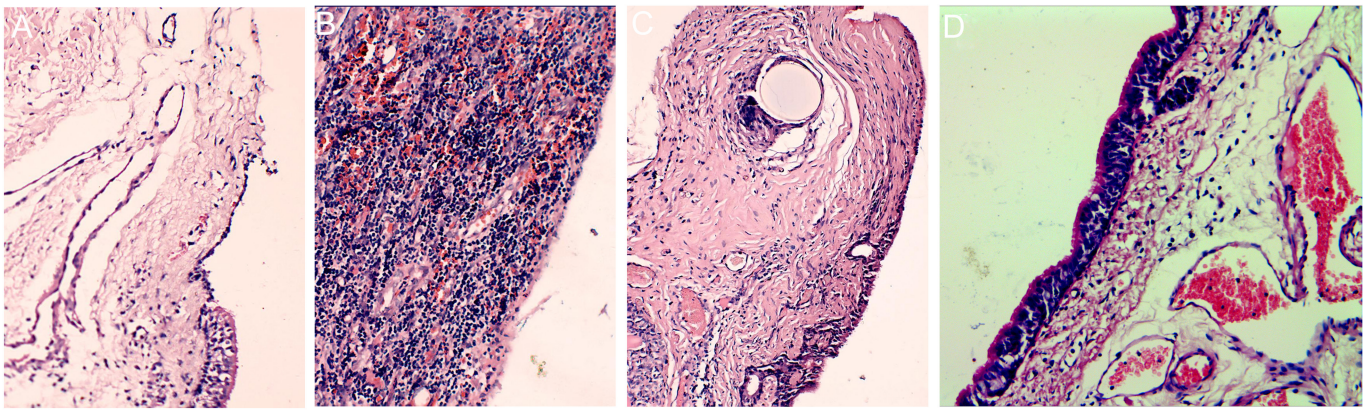

Figure 3. Optical microscope observation. (A) Respiration epithelium interrupted at the cut edge with no sign of epithelial crawling in the control group. (B) Newly generated capillaries were observed in the experiment group. (C) The edge of the ADM is covered by the epithelium. (D) Respiratory epithelium covers all of the ADM.

infectious diseases.[24] Eric et al[25] reported tracheal transplantation in 1 patient with a 20-month follow up. The patient received triple-therapy immunosuppression and returned to work finally, which was a huge breakthrough. However, the transplanted trachea took a long time to restore its functions, and tracheal transplantation was also limited by donor supply. Reconstruction of a structured and functional trachea is still enormous challenge for surgeons.

In this study, ADM was used to repair the trachea defects. In the control group, scar contracture resulted in tracheal stenosis. Contradistinctively, in the experimental group, the airway lumen was well maintained, which confirmed the stiffness of ADM in tracheal reconstruction. Newly generated capillaries were observed 1 week after the surgery, which illustrated that ADM could undergo rapid vascularization. Excellent blood supply could promote wound healing and reduce granulation formation. This may be the considerable reasons for the less scar contracture in the experimental group. At the fourth weekend after the surgery, regenerated epithelium was observed on ADM, and the ADM got completely epithelialized at the eighth weekend after the surgery. However, there was no sign of epithelial crawling in the control group, which indicated that, as a biological scaffold, ADM can promote the migration and repopulation of the respiratory epithelium that guides normal tissue regeneration. Okumura et al[26] reported that once the graft surface was covered by epithelium, the process of stenosis stops. Rapid vascularization and epithelialization of ADM help to maintain the rigid lumen. Thus, the tracheal form and epithelium lining are reconstructed. However, for large tracheal defect repair, mucosal crawling over the construct is even slow and represents a problem. It is believed that the graft containing growth factors may shorten the regeneration time,[27] and ways to speed up the epithelialization of ADM still need to be explored further.

Epithelial regeneration on the graft must be as quickly as possible for the clearance function, which is achieved through epithelial cell growth and differentiation. Epithelial cell crawling primarily covers the repaired region followed by cell differentiation.[28,29] In this study, epithelial regeneration was observed. However, the early regenerated cells were irregular in shape and villus. The ciliated epithelium gradually regenerated and the ADM in cover by normal respiratory epithelium. The regeneration of goblet cells is the evidence of further improvement of a well-functional epithelium, even though it takes a relatively long time. Meanwhile, the mucosal function is recovered. It is reported that application of mesenchyme stem cells on the scaffold would promote epithelial regeneration.[30] However, the combination of the graft with mesenchyme stem cells for tracheal reconstruction needs a certain period of time for a separately cell culture.

Many previous studies have described the use of scaffold that can function as cartilage. Some studies have described the use of absorbable scaffolds such as polyglycolic acid stent or stem cells that will differentiate into cartilage.[31-34] Other studies have described the use of non-absorbable scaffolds.[35] However, some studies have described the reconstruction of small tracheal defects by artificial grafts without scaffold.[10,36]

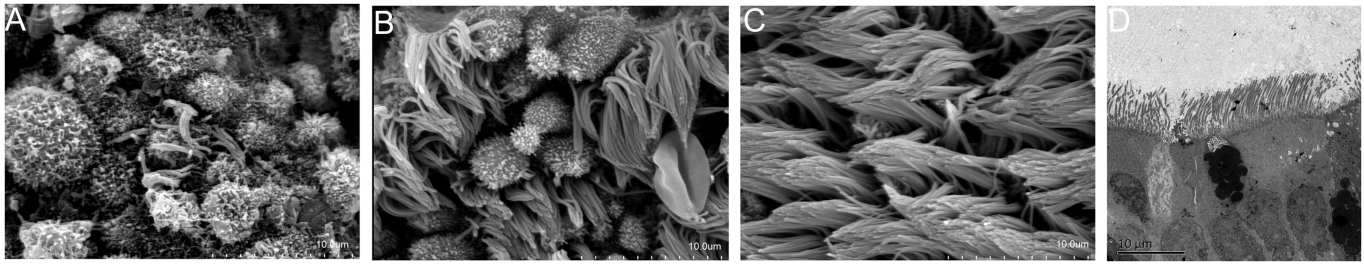

Figure 4. Scanning electron microscope and TEM images of the trachea. (A) The mucosa was regenerated partially with a few irregular cilia. (B) The number of cilia increased and the cilia was more regular. (C) The reconstructed area has been covered by normal ciliated epithelium. (D) Transmission electron microscope images of the restricted area; goblet cells could be observed.

In this study, the tracheal defects were reconstructed with ADM without scaffold, and the trachea maintained its lumen with only mild contracture. However, when repairing a large defect, a stent is always required to maintain the tracheal lumen. Ebihara et al[3] reported tracheal reconstruction with myocutaneous flap without scaffold in 26 cases. The maximum tracheal defects for patients who successfully decannulated without dyspnea were equivalent to 7 tracheal rings and half of the circumference. Since ADM was tightly sutured to the cervical muscle to produce a "muscle flap." We believe that the maximum tracheal defects could be repaired by ADM alone, as reported by Ebihara.[3]

In this study, we reported tracheal defects reconstruction by xeno-ADM, and the trachea maintained its rigid lumen. The membrane graft was quickly vascularized and covered by respiratory epithelium. The regeneration of normal cilia confirmed the clearance function of the trachea. The tracheas were stable with well-functional epithelial lining. As a medical device, this thin membrane would expand the choice of repair materials during tracheal reconstruction.

Data Availability Statement: All the data is available on request from the authors.

Ethics Committee Approval: This study was approved by Ethics committee of Central South University (Approval No: 2019-194, Date: March 11, 2019).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – P. L., XM.Y.; Design – P.L., S.S.L.; Supervision – XM.Y., S.S.L.; Resources – J.K.L., P.L.; Materials – P.L.; Data Collection and/or Processing – P.L., J.K.L.; Analysis and/or Interpretation – P.L.; Literature Search – P.L.; Writing – P.L., S.S.L.; Critical Review – XM.Y.

Declaration of Interests: The authors have no conflict of interest to declare.

Funding: This study received no funding.

References

1. Grillo HC. Tracheal replacement: a critical review. *Ann Thorac Surg*. 2002;73(6):1995-2004. [CrossRef]
2. Czaja JM, McCaffrey TV. The surgical management of laryngotracheal invasion by well-differentiated papillary thyroid carcinoma. *Arch Otolaryngol Head Neck Surg*. 1997;123(5):484-490. [CrossRef]
3. Ebihara M, Kishimoto S, Hayashi R, et al. Window resection of the trachea and secondary reconstruction for invasion by differentiated thyroid carcinoma. *Auris Nasus Larynx*. 2011;38(2):271-275. [CrossRef]
4. Ch'ng S, Wong GL, Clark JR. Reconstruction of the trachea. *J Reconstr Microsurg*. 2014;30(3):153-162. [CrossRef]
5. Ishida I, Oura H, Niikawa H, Handa M. Non-circumferential tracheal resection with muscle flap reconstruction for adenoid cystic carcinoma. *Gen Thorac Cardiovasc Surg*. 2012;60(9):603-606. [CrossRef]
6. Deng D, Xu F, Liu J, et al. Clinical application of pedicled thoracoacromial artery perforator flaps for tracheal reconstruction. *BMC Surg*. 2020;20(1):299. [CrossRef]
7. Neville WE, Bolanowski JP, Kotia GG. Clinical experience with the silicone tracheal prosthesis. *J Thorac Cardiovasc Surg*. 1990;99(4):604-612; discussion 612-613. [CrossRef]
8. Lee DY, Park SA, Lee SJ, et al. Segmental tracheal reconstruction by 3D-printed scaffold: pivotal role of asymmetrically porous membrane. *Laryngoscope*. 2016;126(9):E304-E309. [CrossRef]
9. Choi HS, Suh H, Lee JH, et al. A polyethylene glycol grafted bilayered polyurethane scaffold: preliminary study of a new candidate prosthesis for repair of a partial tracheal defect. *Eur Arch Otorhinolaryngol*. 2008;265(7):809-816. [CrossRef]
10. Kwon SK, Song JJ, Cho CG, et al. Tracheal reconstruction with asymmetrically porous polycaprolactone/pluronic F127 membranes. *Head Neck*. 2014;36(5):643-651. [CrossRef]
11. Xiao Q, Cetto R, Doorly DJ, et al. Assessing changes in airflow and energy loss in a progressive tracheal compression before and after surgical correction. *Ann Biomed Eng*. 2020;48(2):822-833. [CrossRef]
12. Wang J, Zhang H, Sun Y, Liu P, Li S, Cui P. Mechanical properties of de-epithelialized tracheal allografts. *J Thorac Dis*. 2021;13(2):1066-1074. [CrossRef]
13. Wainwright DJ. Use of an acellular allograft dermal matrix (AlloDerm) in the management of full-thickness burns. *Burns*. 1995;21(4):243-248. [CrossRef]
14. Maxwell GP, Gabriel A. Acellular dermal matrix for reoperative breast augmentation. *Plast Reconstr Surg*. 2014;134(5):932-938. [CrossRef]
15. Shi LJ, Wang Y, Yang C, Jiang WW. Application of acellular dermal matrix in reconstruction of oral mucosal defects in 36 cases. *J Oral Maxillofac Surg*. 2012;70(11):e586-e591. [CrossRef]
16. Li P, Li S, Yang X, Tang Q, He X, Wang S. Application of xenogenic acellular dermal matrix in reconstruction of oncological hypopharyngeal defects. *Eur Arch Otorhinolaryngol*. 2016;273(12):4391-4396. [CrossRef]
17. Li P, Li S, Tang Q, et al. Reconstruction of human oncological tracheal defects with xenogenic acellular dermal matrix. *Auris Nasus Larynx*. 2017;44(2):237-240. [CrossRef]
18. Sprague W. Arrive guidelines 2.0. *Vet Clin Pathol*. 2020;49(3):378-379. [CrossRef]
19. Du XF, Kwon SK, Song JJ, Cho CG, Park SW. Tracheal reconstruction by mesenchymal stem cells with small intestine submucosa in rabbits. *Int J Pediatr Otorhinolaryngol*. 2012;76(3):345-351. [CrossRef]
20. Cheng AT, Backer CL, Holinger LD, Dunham ME, Mavroudis C, Gonzalez-Crussi F. Histopathologic changes after pericardial patch tracheoplasty. *Arch Otolaryngol Head Neck Surg*. 1997;123(10):1069-1072. [CrossRef]
21. Almadori G, Di Cintio G, De Corso E, et al. The usefulness of the IMAP propeller flap for trachea and tracheostome reconstruction after resection of parastomal recurrence of squamous cell carcinoma following salvage total laryngectomy. *Eur Arch Otorhinolaryngol*. 2021;278(2):499-507. [CrossRef]
22. Fukunaga Y, Sakuraba M, Miyamoto S, et al. One-stage reconstruction of a tracheal defect with a free radial forearm flap and free costal cartilage grafts. *J Plast Reconstr Aesthet Surg*. 2014;67(6):857-859. [CrossRef]
23. Gao B, Jing H, Gao M, et al. Long-segmental tracheal reconstruction in rabbits with pedicled Tissue-engineered trachea based on a 3D-printed scaffold. *Acta Biomater*. 2019;97:177-186. [CrossRef]
24. Nakamura R, Tani A, Yoshie S, et al. Heparin cross-linked collagen sponge scaffolds improve functional regeneration of rat tracheal epithelium. *J Tissue Eng Regen Med*. 2017;11(11):3027-3037. [CrossRef]
25. Eric M, Timothy H, Benjamin M. Vascularized tracheal transplantation: a twenty month follow up. *Laryngoscope*. 2022;00:1-7.
26. Okumura N, Nakamura T, Natsume T, Tomihata K, Ikada Y, Shimizu Y. Experimental study on a new tracheal prosthesis made from collagen-conjugated mesh. *J Thorac Cardiovasc Surg*. 1994;108(2):337-345. [CrossRef]

27. Kong X, Fu J, Shao K, Wang L, Lan X, Shi J. Biomimetic hydrogel for rapid and scar-free healing of skin wounds inspired by the healing process of oral mucosa. *Acta Biomater*. 2019;100:255-269. [CrossRef]
28. Laplante AF, Germain L, Auger FA, Moulin V. Mechanisms of wound reepithelialization: hints from a tissue-engineered reconstructed skin to long-standing questions. *FASEB J*. 2001;15(13):2377-2389. [CrossRef]
29. Geer DJ, Swartz DD, Andreadis ST. In vivo model of wound healing based on transplanted tissue-engineered skin. *Tissue Eng*. 2004; 10(7-8):1006-1017. [CrossRef]
30. Shin YS, Choi JW, Park JK, et al. Tissue-engineered tracheal reconstruction using mesenchymal stem cells seeded on a porcine cartilage powder scaffold. *Ann Biomed Eng*. 2015;43(4):1003-1013. [CrossRef]
31. Imaizumi M, Nomoto Y, Sato Y, et al. Evaluation of the use of induced pluripotent stem cells (iPSCs) for the regeneration of tracheal cartilage. *Cell Transplant*. 2013;22(2):341-353. [CrossRef]
32. Tsukada H, Majid A, Kent MS, Ernst A, DeCamp MM, Gangadharan SP. Two-stage end-to-end reconstruction of long-segment tracheal defects with a bioabsorbable scaffold grafting technique in a canine model. *Ann Thorac Surg*. 2012;93(4):1088-1092. [CrossRef]
33. Hiwatashi S, Nakayama Y, Umeda S, Takama Y, Terazawa T, Okuyama H. Tracheal replacement using an in-body tissue-engineered collagenous tube "BIOTUBE" with a biodegradable stent in a beagle model: a preliminary report on a new technique. *Eur J Pediatr Surg*. 2019;29(1):90-96. [CrossRef]
34. Dang LH, Tseng Y, Tseng H, Hung SH. Partial decellularization for segmental tracheal scaffold tissue engineering: a preliminary study in rabbits. *Biomolecules*. 2021;11(6) [CrossRef]
35. Park JH, Park JY, Nam IC, et al. Human turbinate mesenchymal stromal cell sheets with bellows graft for rapid tracheal epithelial regeneration. *Acta Biomater*. 2015;25:56-64. [CrossRef]
36. Gubbels SP, Richardson M, Trune D, Bascom DA, Wax MK. Tracheal reconstruction with porcine small intestine submucosa in a rabbit model. *Otolaryngol Head Neck Surg*. 2006;134(6):1028-1035. [CrossRef]