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Original Research

Decellularized Tracheal Allograft in Micro-Miniature Pig: One Year Observation

Michinobu Ohno[1]*, Yasushi Fuchimoto[2], Masataka Higuchi[3], Tetsuji Yamaoka[4], Makoto Komura[5], Akihiro Umezawa[6], Huai-Che Hsu[7], Shin Enosawa[8], Tatsuo Kuroda[9]

[1]*Michinobu Ohno, Department of Pediatric Surgery, Saitama City Hospital, Japan*

[2]*Yasushi Fuchimoto, Department of Pediatric Surgery, International University of Health and Welfare, Japan*

[3]*Division of Pulmonology, Department of Medical Specialties, National Center for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan*

[4]*Department of Biomedical Engineering, National Cerebral and Cardiovascular Center Research Institute, 6-1 Kishibe-Shimmachi, Suita, Osaka 564-8565, Japan*

[5]*Department of Pediatric Surgery, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan*

[6]*Department of Reproductive Biology, National Center for Child Health and Development, Tokyo, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan*

[7,8]*Division for Advanced Medical Sciences, National Center for Child Health and Development, Tokyo, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan*

[9]*Department of Pediatric Surgery, Keio University School of Medicine, 35 Shinanomachi, Shinjyuku-ku, Tokyo 160-8582, Japan*

***Corresponding author**

Michinobu Ohno, Department of Pediatric Surgery, Saitama City Hospital, 2460, Mimuro, Midori-ku, Saitama-shi, Saitama 336–8522, Japan; Tel: +81-48-873-4111; Fax: +81-48-873-4168; Email: mohnomohno2000@hotmail.co.jp

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Abstract

Purpose: Tracheal restoration is an essential treatment for tracheal congenital abnormalities, cancer or injury. We explored the potential of decellularized trachea to restore the trachea in swine, aiming on pediatric application.

Method: We compared pigs with the allogeneic decellularized tracheal graft patch and those with the autotransplanted tracheal patch.

Result: Distortion of the tracheal lumen was observed in both cases. No respiratory symptoms appeared during observation. Histologically, the foci of cartilage regeneration from the recipient trachea was demonstrated in the specimens, suggesting the induction of tracheal reconstitution.

Conclusion: Allogeneic decellularized tracheal grafts could serve as a feasible tracheal restoration, especially for pediatric patients.

ABBREVIATIONS

HE: Hematoxylin and Eosin

INTRODUCTION

Tissue-engineered tracheal implantation caused by many different problems is challenging in the field of laryngeal and thoracic surgery. Pediatric and adult tracheal problem is congenital or postintubation tracheal stenosis, tracheomalacia, closure of tracheostomy, tracheal neoplastic diseases and trauma [1, 2]. Especially, regenerative therapy plays an important role for pediatric diseases. For diseases involving cartilage, such as subglottic stenosis, regeneration and implantation of cartilage are very difficult. We challenged transplantation of trachea in porcine using decellularized trachea by means of hydrostatic pressurization.

MATERIALS AND METHODS

Animals

Porcine tracheal tissues were purchased from Tokyo Shibaura Zouki Co. Ltd. (Tokyo, Japan) for use as an experimentalmaterial. The donor animals were domestic pigs (approximately6–8 months of age). Recipient male pigs (4 weeks old, weighing 7–8 kg) were purchased from Tokyo Laboratory Animals Science Co., Ltd. (Tokyo, Japan).

Decellularization of trachea

Decellularization was performed using a high hydrostatic pressure technique, as reported previously [3-6]. DNA quantification: DNA from decellularized or fresh trachea tissues (20 mg each) was isolated with a DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA).

Surgical procedure

The pigs were placed in a supine position. A midline incision was made, and the cervical trachea was exposed. The area of the three tracheal rings beneath the cricoid cartilage was dissected (Figure 1a). We anastomosed decellularized or fresh tracheal grafts (a fresh trachea is a host native trachea that has been excised and anastomosed again.), adjusted in size to cover the defect, were anastomosed with interrupted 4-0 PDS-II (Johnson & Johnson, NJ, USA) circumferentially (Figure 1b).

Estimation of tracheal grafts

One year after grafting, recipient pigs were anesthetized as described above, and bronchoscopy was performed. The animals were then euthanized with an intravenous injection of potassium chloride and tracheas were excised.

Histological studies

Engrafted areas with decellularized and fresh tracheas were removed, fixed with 10% formalin. The specimens were stained with hematoxylin and eosin (HE), or other stains [6, 7].

Ethical considerations

The institutional animal ethics committee of the National Center for Child Health and Development approved all experimental procedures.

RESULTS

Table 1 summarizes the characteristics observed after the engraftment of decellularized and fresh native tracheas. Decellularized tissue contained only 29.0ng DNA/mg wet tissue, compared with fresh native tissue contained 1175.6ng DNA/mg. Tracheal distortion were less severe in both.Bronchoscopic findings illustrate the area grafted with decellularized trachea and fresh trachea (Figure2a and 2b) maintained the structure of the lumen, but both tracheal lumens showed changes in shape and deformation was observed in both.Macroscopic observation of the grafted area of the trachea shows tracheal narrowing and stenosis were mild in the decellularized trachea group (data were not shown).Assessment of tracheal sections and immunohistochemical staining demonstrates regeneration of recipient chondrocytes was observed under the inner surface of the implanted decellularized trachea in HE sections ((Figure 3a and 3b) magnified area of the square). The donor's recipient chondrocytes appeared to be partially fused with the recipient's trachea. (Figure 3c)

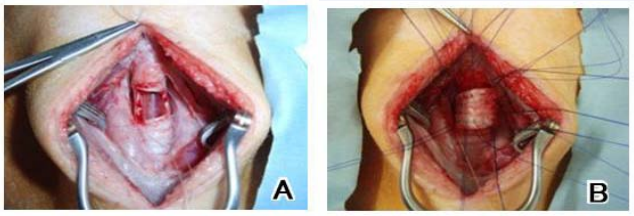

Figure 1 Operative procedure.
(A) Tracheal resection. Three tracheal rings were dissected below the cricoid cartilage with one-third or a semicircle of the tracheal ring.
(B) Tracheal graft patches on dissected defect. The donor tracheal graft was adjusted in size to cover the defect.

Table 1: Summary of results from engraftment of decellularized and fresh trachea tissue.

	DNA Concentration (ng/mg)	Tracheal distortion	Regeneration of chondrocyte
Decellularized trachea	29.0	+	++
Fresh native trachea	1175.6	+	+

DISCUSSION

Macchiarini et al. reported the clinical transplantation of the decellularized trachea for tracheomalacia [8]. The decellularized trachea was made of cadaver trachea seeded with recipient epithelial cells and mesenchymal stem cell derived chondrocytes. Their report is a first human clinical transplantation, however, he was accused of scientific misconduct by some cases [9]. The problem of tissue-engineered trachea is angiogenesis and granulation of the anastomotic site and tracheal growth. Angiogenesis supply nutrients and oxygen, and this process takes months to entire tracheal graft. In case of long length of transplanted trachea, inadequate vascularization results in necrotic changes of the grafted trachea. Granulation hyperplasia occurs at the anastomotic site of the regenerated trachea, causing

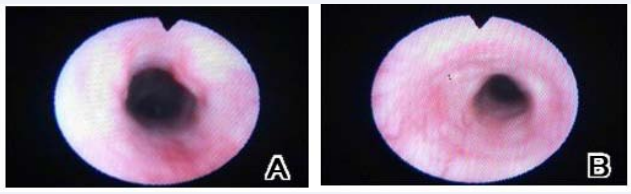

Figure 2 Bronchoscopy findings.
(A) Recipient pigs one year after engraftment of fresh native pig
(B) Recipient pigs one year after engraftment of decellularized pig
Both patches were mild deformation.

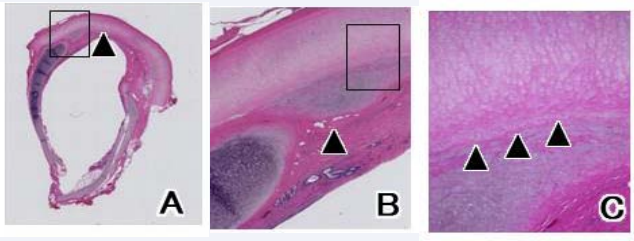

Figure 3 Histopathology of decellularized tracheal patch.
(A) Microscopic observation of decellularized trachea after transplantation in one year, using hematoxylin and eosin. Arrowhead indicates decellularized tracheal grafts.
(B) Magnified views of the framed area in A. Regenerated chondrocytes were founded. Arrowhead indicates regenerating tracheal chondrocytes.
(C) Magnified views of the framed area in B. A foci of chondrocyte fused recipient cartilage. Arrowhead indicates the recipient chondrocyte and the donor chondrocyte appear to be fused.

stenosis of the lumen. Delaere et al. transplanted tracheal graft in subcutaneous tissue of the patient's left forearm [10]. They succeed allotransplantation of this vascularized trachea because the rejection was mild and tracheal cartilage prevents the severe immune response at the patients' arm. They also speculated a wound healing process at the anastomotic site leads to stenosis. We also faced this problem. Anastomotic stenosis was observed in long-term cases in both fresh trachea and decellularized trachea. Tracheal stenosis was popularly observed by one month after transplantation of the circular regenerated trachea into animals [11-13]. We consider that preventing a circumferential anastomosis on the same plane, such as slide tracheoplasty, prevents more appropriate anastomosis from stenosis in clinical situation [14, 15].Especially for the pediatric patients, the growth of decellularized trachea is a critical problem. Our trachea had stenosis and mild rejection as an adverse event without other problem. Hamilton et al. [16] reported an increased in the diameter at the transplant trachea.

Cartilage regeneration was observed in the form of lining the inside of the scaffold and some cartilage grown from the recipient was fused with the donor cartilage. We could not confirm the fused cartilage was really derived from the recipient. Further investigation is needed. Despite many faced problems, the decellularized tracheal graft seems to be the feasible scaffold for clinical tracheal transplantation as its complicated shape, maintaining the extracellular matrix and cell functionality.

CONCLUSION

Tracheal replacement is very challenging because its complicated shape and function. A tissue-engineered decellularized trachea is one of the feasible options that offer many potentials to restore human tracheal disease.

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