Mini Review

A regenerative approach for partial tracheal defects, an *in vivo* canine model

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In the field of head and neck surgery, tracheal resection is frequently required for patients with cancer or trauma. There are several approaches for reconstructing the tracheal wall, but almost all require repeated skilled surgeries, which intend to fill the defect and create new airway space using autologous or artificial grafts.

Regenerative medicine has made remarkable progress and has been applied clinically in some organs. Thus in this study, the usefulness of a tissue engineering approach for tracheal reconstruction was evaluated. A partial defect was created in canine cervical tracheas. A scaffold made of polypropylene and collagen sponge was sutured at the defect site. Postoperative status was evaluated by endoscopy, radiography, and histology. In all five cases, epithelialization of the scaffold luminal surface was observed without deformity or complications. Histological data also supported the functional regeneration of the trachea using this approach. This simple tissue engineering approach is a good method for reconstruction of the trachea with partial defects.

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Introduction

There are many approaches to reconstruct the trachea after partial resection, but no optimal method has yet been established.

The "Trough" method is widely used as a standard tracheal reconstructive technique[1,2], but repeated skilled surgeries are usually required. Thus, patients have cosmetic, financial and mental obstacles for a long time. Although artificial materials have also been used since the 1970's[3] to cover or mold the defect, it is difficult to obtain hybrid tissue, host tissue and biomedical material *in vivo*. Reasons for insufficient outcomes with conventional methods include lack of epithelium, reconstructed site deformities and infection.

Therefore, to solve these problems in this study, *in situ* tissue engineering technique was introduced for a partial tracheal reconstruction model using a scaffold made of polypropylene and collagen. Our technique is more useful than other procedures, because it requires only a single and easy operation for the scaffold to be covered with living tissue *in vivo*.

Materials and Methods

1)Scaffold preparation

A single sheet of knitted polypropylene with a pore size of 260 μm (Marlex Mesh; CR Bird, Inc., Billerica, MA, USA) was rolled and reinforced with spiral fine polypropylene strings (Fig.1A). A 1% aqueous solution (pH 3) of porcine dermal atelocollagen, comprised of type I and III (Nippon Meatpackers, Inc., Ibaraki, Japan), was applied on both sides of this scaffold framework. After coating, the scaffold was freeze dried (FDU-810, Tokyo Rikakikai Co. Ltd., Tokyo, Japan) and cross-linked with a vacuum dry oven (VOS-300SD, Tokyo Rikakikai Co. Ltd., Tokyo, Japan) (Fig.1B). This spongy collagen accelerates cellular attachment and ingrowth in the scaffold.

2)Animals and surgical procedures

Animal care, housing and experimental procedures were conducted under the Guideline for Animal Experiments of Kyoto University. Five beagle dogs weighing 10 to 12 kg were used. Under anesthesia with subcutaneous injections of ketamine hydrochloride (5.0 mg/kg; Sankyo Co., Ltd, Tokyo, Japan) and xylazine hydrochloride (2.0 mg/kg; Bayer, Ltd., Tokyo, Japan), the cervical trachea of each dog was exposed by sterilized instruments. A round resection, 1.5-2.0 cm in diameter was created by a scalpel, preserving the marginal mucosa (Fig.2A). The above mentioned artificial scaffold was trimmed based on the parameters of the cartilage defect (Fig.2B). The implant was preclotted with peripheral blood to prevent postoperative air leakage, and then was sutured to the resected portion with 3-0 bioabsorbable sutures (Vicryl; Ethicon, Inc., Someville, NJ, USA) (Fig.2C). Operated site was closed and disinfected. Postoperative general status of each dog was checked carefully and periodically. Ampicillin sodium (Meiji Seika Kaisya Ltd., Tokyo, Japan) was administered by subcutaneous injection (250 mg) for three days, followed by oral application (500 mg/day) for 1 week to prevent postoperative infection. To harvest the operated site,

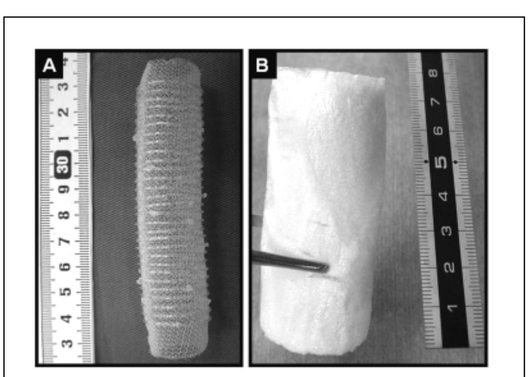

Fig.1
(A) Polypropylene framework of scaffold.
(B) Appearance of scaffold with spongy collagen.

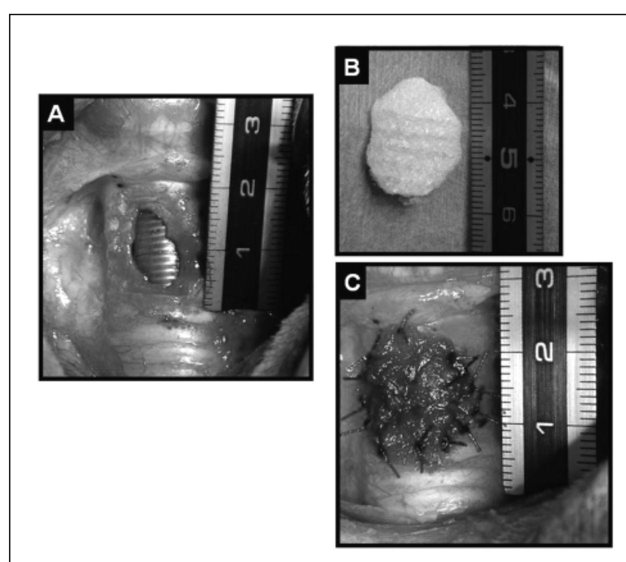

Fig.2
(A) A tracheal defect created on anterior wall.
(B) Trimmed scaffold.
(C) Scaffold preclotted with blood was fixed to the defect boundaries with bioabsorbable sutures.

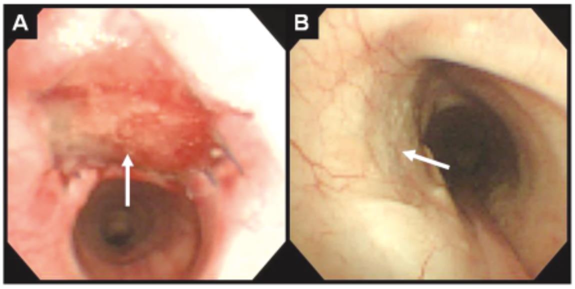

Fig.3 The arrows indicate the operated sites
(A) Luminal surface was covered with soft tissues on day 7 after the operation.
(B) An image 1 year after operation in another dog. Implant was completely covered with regenerated mucosa without any granulation or displacement. Capillary vessels are also seen in the regenerated mucosa.

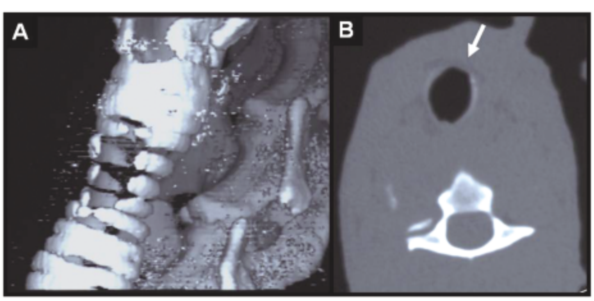

Fig.5 Computed tomographic images taken 1 year after the surgery
(A) Three-dimensional reconstructed image from fine axial sections indicates no obvious new cartilage formation.
(B) An axial image of the operated site shows a smooth contour of the luminal surface. The arrow indicates the operated site.

the animals were humanely sacrificed using cardiac injection of pentobarbital sodium (400 mg; Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan) under above mentioned anesthesia.

3)Postoperative evaluation

Postoperative status was periodically evaluated by endoscopy, radiography and histology. Endoscopic observation was performed under sedation with ketamine hydrochloride and xylazine hydrochloride (same concentrations as described previously) to monitor the regenerative status of the tracheal lumen with a video-endoscopy system consisting of a bronchoscope (BF type 1T240,

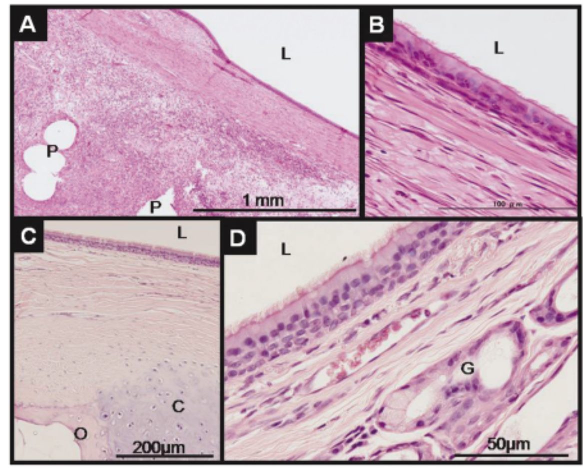

Fig.4 Hematoxylin-eosin stained images of the operated site
(A) An image taken at one month after the operation. A lot of inflammatory cells are observed submucosal area. P: polypropylene, L: tracheal lumen.
(B) A magnified image indicates regenerated cilia with a low concentration.
(C) An image of the operated site 8 months after the surgery. Regenerated cartilage and pseudo-ossification are seen. C: cartilage, O: pseudo-ossification.
(D) Submucosal glands are also observed in a specimen 8 months after the operation. Cilia are well-regenerated. G: gland.

Olympus Co., Tokyo, Japan) and a video processor (CV-240, Olympus Co., Tokyo, Japan). Histological assessments were performed using a light microscope.

One year after the operation, radiographical evaluation was made with a helical CT scanner system (Legato Duo, GE Yokokgawa Medical Systems, Tokyo, Japan).

Results

All dogs did well during the observation periods. None showed any sign of infection or complication. Endoscopic examinations revealed that epithelialization started at 1 week postoperatively without any luminal deformity (Fig.3A). After completion of epithelialization, no scaffold exposure was seen up to one year postoperatively (Fig.3B). Histological data showed that epithelialization of the luminal surface was complete (Fig.4A) with submucosal infiltration of inflammatory cells one month after the operation, although the concentration of cilia was still low (Fig.4B). Regenerated immature cartilage, pseudo-ossification, and subepithelial glands were observed after 8 months (Fig.4C,D). Well-regenerated cilia were also seen in this time point. Computed tomographic images showed no obvious neo-cartilage (Fig.5A), but the contour of the luminal surface at the operated site was smooth (Fig.5B).

Discussion

Regenerative medicine has been clinically employed in some organs. To date, we have applied *in situ* tissue engineering techniques for mastoid air cells[4], cricoid cartilage[5] and nerves[6,7] by using artificial scaffolds in human subjects. This study, therefore, examined whether a tracheal tissue engineering approach could be applied clinically.

Tracheal reconstruction has been performed for more than 50 years[8], but no ideal technique has been established. Surgeons still encounter insufficient results with tracheal reconstruction due to the lack of ciliated epithelium, which contributes to autopurification, infection or foreign body reaction, and repeated skilled surgeries[2,9] including tracheostomy.

In the current study, a scaffold made of polypropylene and porcine-derived atelocollagen was used to solve the above mentioned problems. Polypropylene is widely used as non-bioabsorbable plastic material and has high biocompatibility. This material has already been used clinically for abdominal surgeries. The porcine-derived atelocollagen has also been tested for clinical use in terms of stability and safety. The mechanical power against compression of this scaffold has also been checked by Omori K et al.[5]. They proved it had almost the same strength as canine trachea. As polypropylene is synthetic plastic material, the strength and size of our scaffold are easily manipulated by modifying its structure. The advantages of using this material are these safety and pliability.

Using the same kind of artificial tracheal tube, regeneration of the trachea after circumferential resection was attempted in the canine model by Okumura N et al.[10]. They had favorable results, including cellular invasion of the scaffold, epithelialization of the luminal surface, and complete integration of the scaffold with the recipient tissue. Thus, we applied the scaffold to human head and neck surgeries. The first human case was reported by Omori K et al.[11]. In spite of good results in the previous canine model with circumferential resection, the delayed epithelialization and deformity of the luminal surface of scaffold was observed as a postoperative problem within 2 months in the human patient with a partial tracheal resection. Since partial tracheal resection is frequently performed in head and neck surgeries for malignancy or injury, these problems should be resolved *in vivo*. The present procedure is revised, in which the inner mucosa was preserved at the margin of the tracheal defect. It prevents dislocation of the scaffold and accelerates epithelial ingrowth over the inner surface.

Recently, tracheal regeneration for similar partial tracheal defects was attempted using small intestine submucosal tissue in rabbit models[12,13]. They both showed nice epithelialization data histologically, but further studies were warranted to obtain long term results without granulation or stenosis.

The results of this study were extremely positive, especially in the early regeneration of ciliated stratified epithelium without scaffold dislocation. Newly formed immature cartilage tissue and pseudo-ossification were also observed. These may contribute to the mechanical strength of the regenerated tracheal tissue. However, to achieve a structured and layered tracheal regeneration with mature cartilage and muscle, the addition of cells and/or regulation factors should be considered[14-17]. Once this hurdle has been cleared, the framework could be changed into a bioabsorbable one. Then, this approach may be applicable to infant patients. Other tissue engineering techniques should be also considered for larger defects, i.e. creating tailored trachea *ex vivo* before a surgical resection.

The results of this study support the feasibility of our novel approach for clinical use, although further studies are warranted. This tracheal regenerative approach could become the technique of choice as it is simple, cost-effective and less invasive.

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