underwent a canal wall down tympanomastoidectomy operation. Fifteen of these patients underwent a new method in which the open cavity was lined with a pedicle periosteal flap of the postauricular region together with free temporal fascia grafts (group I), and as a control, 10 patients underwent the standard operation that uses only free temporal fascia grafts (group II).

All procedures are shown in Figures 1–4. In all cases, T-shaped external auditory canal plasty was done during the first stage of the operation. After the canal wall-down mastoidectomy had been performed,

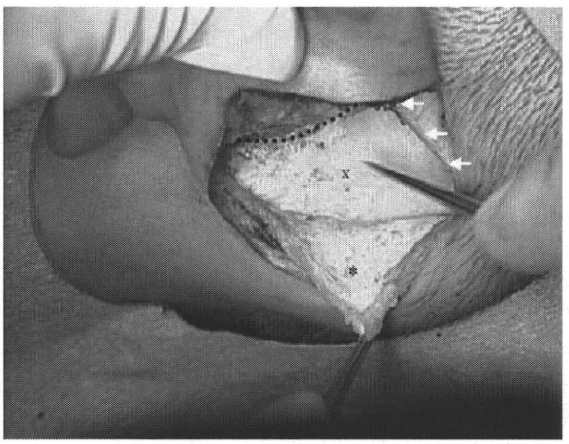

Figure 1. Skin incision and flap. White arrows, temporal line; dotted line, posterior wall of the external auditory meatus; X, mastoid cortex; asterisk, subcutaneous tissue over the mastoid cortex.

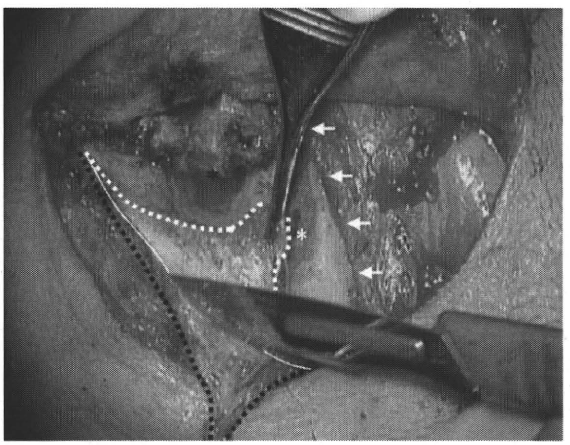

Figure 2. Preparation of the upper and lower layers. Subcutaneous tissue over the mastoid cortex is separated into two layers with a scalpel along the white line. Black and white dotted lines indicate the border of the upper and the lower layers of the subcutaneous tissue, respectively. White arrows, temporal line; black asterisk, tympanic membrane; white asterisk, mastoid cavity.

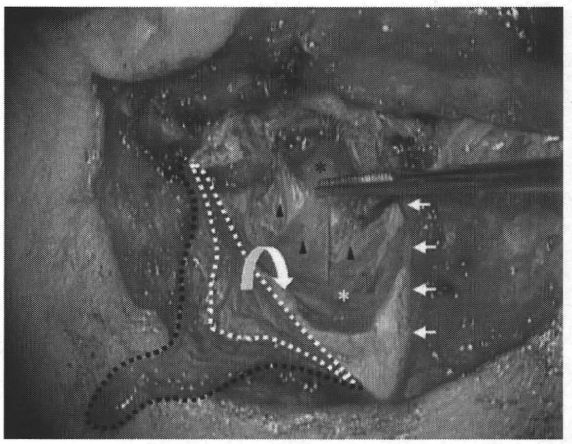

Figure 3. Combination of the periosteal flap and the free temporal fascia grafts. White dotted line, lower layer (periosteal flap); black dotted line, upper layer; white arrows, temporal line; black asterisk, tympanic membrane; white asterisk, mastoid cavity; black arrowheads, free temporal fascia grafts.

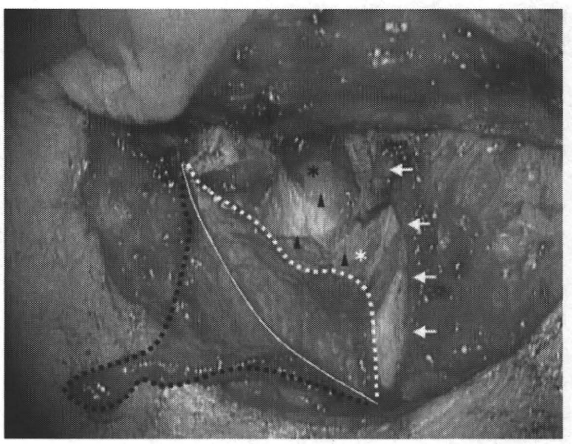

Figure 4. No bone exposed in the region of the opened mastoid cavity. The posterior wall of the open cavity was lined with the periosteal flap. There is no bone exposed in the region of the tympanomastoid cavity. White dotted line, lower border of periosteal flap; black dotted line, upper layer; white line, border of the upper and the lower layer; white arrows, temporal line; black asterisk, tympanic membrane; white asterisk, mastoid cavity; black arrowheads, free temporal fascia grafts.

the tympanic membrane was repaired with temporal fascia grafts. In group I, the subcutaneous tissue over the mastoid cortex was separated into two layers with a scalpel (Figure 2). The upper layer was composed of the subcutaneous tissue and the lower layer was the periosteal flap. Ablation of the subcutaneous tissue beyond the posterior boundary line of the mastoidectomy had to be performed before this procedure to obtain a sufficient periosteal flap and to improve its

mobility. The posterior wall of the open cavity was lined with this periosteal flap and the remaining bone-exposed region was completely lined with the free temporal fascia grafts, which were fixed by fibrin glue (Figure 4). The upper layer was used to cover the open cavity of the postauricular region.

The results of both groups were compared as regards three items, as follows. Item 1: were any bone-exposed regions present in the newly formed external auditory meatus on the first day that the tampon gauze was exchanged postoperatively? Item 2: how many days did the entire surface of the external auditory meatus take to dry? Item 3: for how many days did the patients remain in the hospital after the operation?

Patients were discharged from hospital when they no longer needed to change the tampon gauze in their ears and only needed to apply eardrops.

Results

Table I shows the results of the comparison between groups I and II. For item 1, the tampon gauze was first exchanged an average of 8.5 days after the procedure in all patients. In group I, a bone-exposed region was observed in only one case, which occurred because the transplanted temporal fascia was out of place. This dislocated fascia was immediately returned to its original position. In contrast, bone-exposed regions were observed in all patients in group II. For item 2, it took an average of 30.8 days for the entire surface of the external auditory meatus to dry completely in group I. In group II, it took an average of 81.4 days for it to dry in six patients. Since the remaining four patients had bone-exposed regions, the external auditory meatus could not dry during the observation period. As regards item 3, group II patients remained hospitalized for an average of 10 days longer than patients in group 1.

Table I. Results of the comparison between groups I and II.

Item	Group I (n = 15)	Group II (n = 10)	p value (Student's t test)
1	1/15 (6.7%)	10/10 (100%)	< 0.001
2	30.8 days	81.4 days*	< 0.001
3	18.5 days	29.4 days	< 0.005

Group I, the open cavity was lined with a pedicle periosteal flap of the postauricular region together with free temporal fascia grafts; group II, the standard operation that uses only free temporal fascia grafts. Item 1: bone-exposed rate in the newly formed external auditory meatus on the first day that the tampon gauze was exchanged postoperatively. Item 2: how many days did the entire surface of the external auditory meatus take to dry perfectly? Item 3: for how long did the patients remain in the hospital after the operation?

*In group II, it took an average of 81.4 days for the entire surface to dry in 6 of 10 patients. In the remaining four patients, the external auditory meatus did not dry during the observation period.

Discussion

There are two major approaches for tympanomastoidectomy: canal wall-up and canal wall-down. The former is divided into canal wall-up alone and temporary canal wall-down accompanied by reconstruction of the posterior wall. Each approach has advantages and disadvantages [3–5]. Canal wall-down tympanomastoidectomy is a well-established surgical procedure for the treatment of chronic otitis media and severe cholesteatoma in particular [3,6]. However, the newly formed cavity is far larger than that of the original external acoustic meatus, and consequently, this approach has negative sequelae called cavity problems [1,2]. Subsequent outpatient care is required frequently. Some postoperative complications occur because the bony wall of the open cavity remains partially non-epithelialized, and physiologically abnormal states such as bone-exposed regions make the ear susceptible to infection and relapse. Therefore, early epithelialization of the entire surface of the bony wall is essential for quick healing and for prevention of cavity problems.

It is necessary to provide a sufficient blood supply for the free flap on the bone surface to keep it alive and to prevent infection. If the entire surface of the open cavity is covered with only free temporal fascia, its fascia is too large to prevent necrosis. The maximum boundary that can supply blood from the original external auditory skin may be a fascia graft from the tympanic membrane and its very near surroundings. In this case, we have no choice but to leave the bone exposed in the newly formed posterior wall.

In this study, we epithelialized the entire surface of the bony wall very early in the procedure by using the pedicle periosteal flap of the postauricular region. As this flap has a wide pedicle, it can be supplied with blood by a postauricular artery. It is located in an anatomic region that can supply blood to its surrounding free fascia grafts. Since the periosteum is the contact surface with the bone wall of this flap, a flap covering the bone is more suitable than other flaps. Moreover, this technique is very simple and easy and it does not take so much time for operation.

Most ears were rendered dry and safe, with cavity problems minimized by this simple technique. This technique is also valid in terms of medical economy because it shortens the hospitalization period and subsequent outpatient care is not required frequently [7].

Conclusions

We attempted to completely epithelialize and thereby expedite healing of the surface of the newly formed bony wall using a pedicle periosteal flap of the postauricular region together with free temporal fascia grafts in canal wall-down tympanoplasty. This new operative technique is efficacious to reduce postoperative complications in canal wall-down tympanoplasty and is also valid in terms of medical economy.

Acknowledgment

This study was supported by a Health Sciences Research Grant from the Ministry of Health, Labor and Welfare of Japan.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Saunders JE, Shoemaker DL, McElveen JT Jr. Reconstruction of the radical mastoid. Am J Otol 1992;13: 465–9.

[2] Wormald PJ, Nilssen ELK. The facial ridge and the discharging mastoid cavity. Laryngoscope 1998;108:92–6.

[3] Marco-Algarra J, Gimenez F, Mallea I, Armengot M, de la Fuente L. Cholesteatoma in children: results in open versus closed techniques. J Laryngol Otol 1991;105: 820–4.

[4] Karmarkar S, Bhatia S, Saleh E, DeDonato G, Taibah A, Russo A, et al. Cholesteatoma surgery: the individualized technique. Ann Otol Rhinol Laryngol 1995;104: 591–5.

[5] Sheehy JL. Tympanoplasty with mastoidectomy: present status. Clin Otolaryngol 1983;8:391–403.

[6] Jackson CG, Glasscock ME 3rd, Nissen AJ, Schwaber MK, Bojrab DI. Open mastoid procedures: contemporary indications and surgical technique. Laryngoscope 1985;95: 1037–43.

[7] Blake P, Morrissey G. Canal wall down techniques for managing cholesteatoma. Aust N Z J Surg 1991;61: 914–18.

Acta Oto-Laryngologica, 2010; 130: 79–83

informa
healthcare

ORIGINAL ARTICLE

A tissue-engineering approach for stenosis of the trachea and/or cricoid

SHIN-ICHI KANEMARU[1], SHIGERU HIRANO[2], HIROO UMEDA[3], MASARU YAMASHITA[4], ATSUSHI SUEHIRO[2], TATSUO NAKAMURA[5], TOSHIKI MAETANI[1], KOICHI OMORI[6] & JUICHI ITO[2]

[1]*Department of Otolaryngology-Head and Neck Surgery, Medical Research Institute, Kitano Hospital, Osaka,* [2]*Department of Otolaryngology-Head and Neck Surgery, Graduate School of Medicine, Kyoto University, Kyoto,* [3]*Department of Otolaryngology, Shizuoka Prefectural General Hospital, Shizuoka,* [4]*Department of Otolaryngology, Kusatsu General Hospital, Shiga,* [5]*Department of Bioartificial Organs, Institute for Frontier Medical Sciences, Kyoto University, Kyoto and* [6]*Department of Otolaryngology, Fukushima Medical University School of Medicine, Fukushima, Japan*

Abstract

Conclusion: This new regenerative therapy shows great potential for the treatment of stenosis of the trachea and/or cricoids (STC). *Objectives:* To estimate the potential of tissue-engineered artificial trachea (AT) for treatment of STC in clinical applications. We previously reported that AT was a useful material for implantation into a tracheal defect after resection of cancer. There are many causes of stenosis of the respiratory tract and STC is particularly difficult to treat. *Methods:* The AT was a spiral stent composed of Marlex mesh made of polypropylene and covered with collagen sponge made from porcine skin. Three patients with STC were treated by this tissue-engineering method. All of them suffered from STC caused by long endotracheal intubations. They underwent a two-stage operation. In the first operation, after resection of the stenotic regions, the edge of the tracheal cartilage was sutured to the edge of the skin. The tracheal lumen was exposed and a T-shaped cannula was inserted into the large tracheostoma. At 3 weeks to 2 months after the first operation, the trachea and skin were separated. The trimmed AT with venous blood and basic fibroblast growth factor (b-FGF) was then implanted into the cartilage defect. *Results:* Postoperatively, all patients were able to breathe easily and had no discomfort in their daily activities. Six months after the second operation, we observed enough air space in the trachea and cricoid by computed tomography (CT) imaging and fiber endoscopy.

Keywords: *Respiratory tract, artificial trachea, basic fibroblast growth factor, regeneration of the trachea, staged operation*

Introduction

Stenosis of the trachea and/or cricoids (STC) is a fibrotic narrowing of the airway at the level of the cricoid and/or tracheal cartilage, which can result in severe dyspnea. There are many causes of STC. Post-intubation and tracheostomy are the most common causes of acquired STC. In spite of technological improvements and more skilful patient care in intensive care units, STC still constitutes a serious iatrogenic sequela after intubation and tracheostomy [1–4]. Although management varies according to different concepts and techniques, there is no well established treatment. Following STC, it is very difficult to recreate an airway structure that allows enough space to breathe.

Progressive tissue-engineering techniques have made it possible to regenerate various tissues and/or organs [5]. According to the concept of tissue engineering, three elements – cells, scaffold, and regulatory factors – are essential to regenerate tissues and/or organs. Depending on the in vivo condition,

Correspondence: Shin-ichi Kanemaru MD PhD, Department of Otolaryngology-Head and Neck Surgery, Medical Research Institute, Kitano Hospital, 2-4-20 Ohgimachi, Kita-ku, Osaka, 530-8480, Japan. Tel: +81 6 6312-8824. Fax: +81 6 6312-8867. E-mail: kanemaru@ent.kuhp.kyoto-u.ac.jp

(Received 2 April 2010; accepted 6 April 2010)

ISSN 0001-6489 print/ISSN 1651-2251 online © 2010 Informa Healthcare
DOI: 10.3109/00016489.2010.496462

these three elements are chosen and can be applied in combination [6]. On the basis of this in situ tissue engineering, our group has developed a new artificial trachea (AT) scaffold made from a Marlex mesh tube covered with collagen sponge and has used it successfully to repair tracheal defects after resection of thyroid cancer. In this clinical study, we investigated the application of the new AT scaffold for the treatment of STC post-intubation.

Material and methods

Scaffold

The AT scaffold was composed of a spiral stent and a single sheet mesh (Marlex mesh; CR Bard Inc., Billerica, MA, USA) made of polypropylene and covered with collagen sponge made from porcine dermal atelocollagen (Nippon Meatpackers Inc., Ibaraki, Japan) consisting of type I (70%) and type III (30%) collagen dissolved in a hydrochloric acid solution, pH 3.0, at a concentration of 1.0% (Figure 1). A single sheet mesh has a pore size of 260 μm. After collagen coating, the scaffold was freeze-dried with a freeze dryer (FDU-810; Tokyo Rikakikai Co. Ltd, Tokyo, Japan), and cross-linked with a vacuum dry oven (VOS-300SD; Tokyo Rikakikai Co. Ltd).

Patients and surgical procedures

Three patients with STC were treated by this tissue-engineering method. All of them had suffered from STC caused by endotracheal intubations. Table I shows the patient profiles. This new tissue-engineered treatment for STC was applied to a human in accordance with the IRB guidelines of Kyoto University and Medical Research Institute, Kitano Hospital.

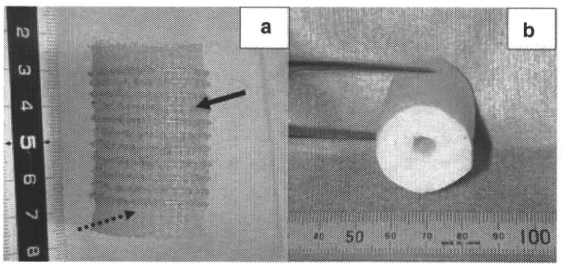

Figure 1. Tissue-engineered artificial trachea (AT) scaffold. (a) Framework of the AT scaffold. Black arrow indicates a spiral stent. Black dotted arrow indicates a single sheet mesh. These materials are made of polypropylene. (b) Framework of the AT scaffold is covered with collagen sponge made from porcine dermal atelocollagen.

All subjects underwent the same two-stage operations. In the first operation, after resection of the stenotic regions, the edge of the tracheal cartilage was sutured to the edge of the skin. The tracheal lumen was exposed and a T-shaped cannula was inserted into the large tracheostoma. At 3 weeks to 2 months after the first operation, the trachea and skin were separated and the AT was trimmed. Venous blood and basic fibroblast growth factor (b-FGF) (Fibrast; Kaken Pharmaceutical Co. Ltd, Tokyo, Japan) was then implanted into the cartilage defect. The artificial material was sutured to the edge of the trachea. Figure 2 shows the operative procedures.

Assessment

Endoscopic examination was performed periodically to observe regeneration of the AT implanted site with a video-endoscope system (BF typeIT 240, CV240, CLV-U40D, Olympus Co., Tokyo, Japan). Whole images of the reconstructed site were estimated by computed tomography (CT) 6 months after the second operation.

Results

Postoperatively, all patients were finally able to breathe easily and had no discomfort in their daily activities. Six months after the second operation, we observed enough air space in the trachea and the cricoid by CT imaging and a fiber endoscope (Figure 3).

Discussion

There are many causes of STC. The most frequent cause of STC is post-intubation. The long-term mechanical stress of intubation causes a deficiency of blood and necrosis of tracheal cartilage. This leads to cicatrization of the necrotic region and results in cicatricial stenosis. Once this chain reaction begins in the tubular trachea, it is very difficult to stop its progression. The rates of STC following tracheostomy and laryngotracheal intubation have been reported to range from 0.6% to 21% and 6% to 21%, respectively [7].

Tracheal resection followed by end-to-end anastomosis is a well-established technique performed under well-established indications. High success rates of over 70% have been reported [1,2,4,7]. However, in cases where long tracheal segments are to be resected, end-to-end anastomosis cannot be adapted. Also, lesions that involve the infraglottic larynx and the upper

Table I. Patient profiles.

Case no.	Age (years)	Sex	Cause of STC	Adverse conditions
1	71	F	EI after traffic accident	Infection (MRSA) Asthma
2	39	F	EI after status asthmaticus	Infection (MRSA) Atopic dermatitis Asthma
3*	45	M	EI after inhalation burn	Infection (MRSA) Autoimmune renal failure

EI, endotracheal intubation; MRSA, methicillin-resistant Staphylococcus aureus; STC, stenosis of the trachea and/or cricoids.
*Patient no. 3 also had stenosis of vocal fold as a complication of inhalation burn.

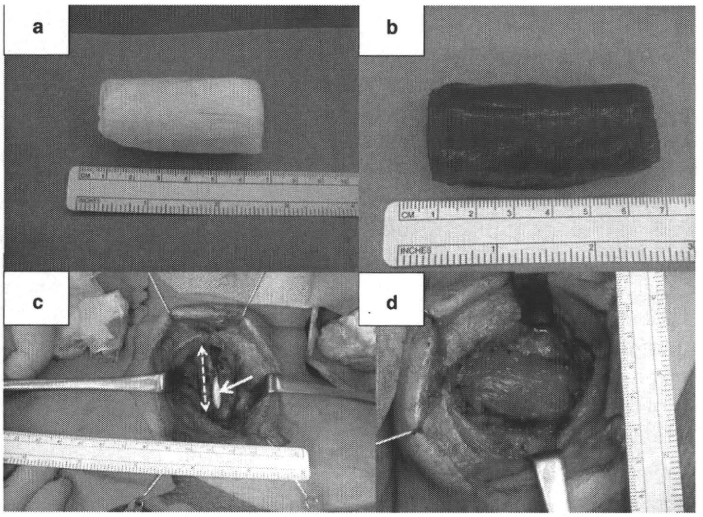

Figure 2. Operative procedure of the second-stage operation. (a) Trimmed artificial trachea (AT) scaffold: AT was trimmed for the size and the shape of the tracheal defect. (b) Trimmed AT scaffold with venous blood and basic fibroblast growth factor (b-FGF). Venous blood and b-FGF were added to the AT immediately before suturing to the trachea. (c) After separation of the trachea and skin. White arrow indicates intubation tube. White dotted arrow indicates the defective region of tracheal cartilage. (d) Trimmed AT scaffold with venous blood and b-FGF was sutured to the defective region of tracheal cartilage with 3-0 absorbable thread.

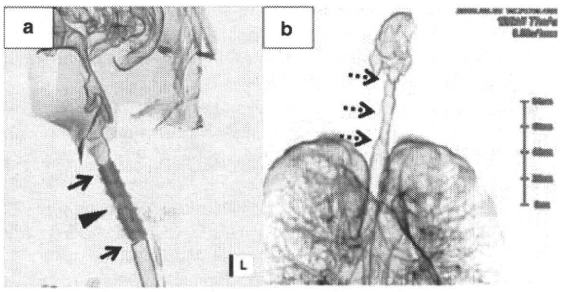

Figure 3. CT scan air-emphasized images of trachea before and after operation. (a) Case no. 2 before operation. A cannula was inserted in the region of STC (black arrows); black triangle indicates the stomal orifice. (b) Case no. 2 after operation. Although the region of STC remains, the inner space of the trachea (black dotted arrows) is maintained without the cannula.

trachea are much more difficult to repair surgically [1–3,7]. In this study, all cases involved long resections of the trachea with cricoid. In case no. 2, it proved particularly difficult to maintain the inner space of the trachea because of necrosis of a long tracheal segment.

Even now, there is no definite and successful treatment for severe cases like those presented here. Major variations in the treatment of STC consist of reconstructive material, the way the operation is performed, and postoperative sequelae. Although autologous tissues such as cartilage are the best reconstructive materials available, it is impossible to obtain them in enough volume and of adequate shape. On the other hand, artificial materials are inferior to autologous tissues as regards affinity and anti-infection. Considering these points, the AT used here is near

to an ideal biomaterial. We have previously reported that our AT had high affinity, sufficient volume, good shape, and mechanical strength equal to normal trachea. Moreover, we confirmed by histological examination that the luminal surface of reconstructed trachea had a normal mucosa with cilia [8–11].

After successful outcomes of animal experiments, we applied AT as a regenerative biomaterial for repairing tracheal defects after resection of cancer in humans [12,13]. These clinical applications succeeded in all cases because most of the trachea itself was normal, except for small cancer invasive regions. However, most patients with STC have adverse conditions, including infections such as methicillin-resistant Staphylococcus aureus (MRSA), asthma, and systemic diseases. The cases presented here have these unfavorable conditions, which prevent the trachea from regenerating.

According to the concept of in situ tissue engineering, for regeneration of tissues/organs it is necessary to arrange three elements – cells, scaffolds, and regulatory factors – in favorable conditions [5,6]. In this clinical study, we arranged two of the elements, scaffold and regulatory factors, because cells were supplied from the host tissue. In addition, to create the best regenerative conditions, we selected a two-staged operation. The purpose of the first operation was to enlarge the region of STC, allow early epithelialization of its luminal side, and prevent infection. After creating these suitable conditions, we implanted AT with b-FGF in the second operation. This two-staged operation may be a good strategy for severe cases of STC.

Basic FGF is a regulatory factor that plays an important role in regeneration of the trachea. In normal tissues, b-FGF is present in basement membranes and in the subendothelial extracellular matrix of blood vessels [14]. It stays membrane-bound as long as there is no signal peptide [15]. It has been hypothesized that during wound healing of normal tissues or during tumor development, the action of heparan sulfate-degrading enzymes activates b-FGF and mediates the formation of new blood vessels, a process known as angiogenesis [14–16]. Administration of b-FGF may therefore be effective in early stages of the regenerative process [17–19]. Collagen sponge, which is a component of AT, could provide not only inducer for cell migration from host tissue but also sustained release of b-FGF [20]. Basic FGF may be not suitable to repair tracheal defects after resection of cancer as it may contribute to tumor recurrence, but there is no such risk in the treatment of STC caused by prolonged intubations [16].

Conclusions

We applied tissue-engineered AT and b-FGF for the treatment of STC [2]. A two-stage operation for the treatment of severe STC may provide better regenerative conditions [3]. This new regenerative therapy shows great potential for the treatment of STC.

Acknowledgment

This study was supported by a Health Sciences Research Grant from the Ministry of Health, Labor and Welfare of Japan.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Anand VK, Alemar G, Warren ET. Surgical considerations in tracheal stenosis. Laryngoscope 1992;102:237–43.
[2] Maddaus MA, Toth JL, Gullane PJ, Pearson FG. Subglottic tracheal resection and synchronous laryngeal reconstruction. J Thorac Cardiovasc Surg 1992;104:1443–50.
[3] Couraud L, Jougon JB, Velly JF. Surgical treatment of non-tumoral stenoses of the upper airway. Ann Thorac Surg 1995; 60:250–60.
[4] Grillo HC, Donahue DM, Mathisen DJ, Wain JC, Wright CD. Postintubation tracheal stenosis. Treatment and results. J Thorac Cardiovasc Surg 1995;109:486–93.
[5] Bianco P, Robey PG. Stem cells in tissue engineering. Nature 2001;414:118–21.
[6] Zdrahala RJ, Zdrahala IJ. In vivo tissue engineering: Part I. Concept genesis and guidelines for its realization. J Biomater Appl 1999;14:192–209.
[7] Omori K, Nakamura T, Kanemaru S, Asato R, Yamashita M, Tanaka S, et al. Regenerative medicine of the trachea: the first human case. Ann Otol Rhinol Laryngol 2005;114:429–33.
[8] Omori K, Tada Y, Suzuki T, Nomoto Y, Matsuzuka T, Kobayashi K, et al. Clinical application of in situ tissue engineering using a scaffolding technique for reconstruction of the larynx and trachea. Ann Otol Rhinol Laryngol 2008; 117:673–8.
[9] Sarper A, Ayten A, Eser I, Ozbudak O, Demircan A. Tracheal stenosis after tracheostomy or intubation: review with special regard to cause and management. Tex Heart Inst J 2005;32:154–8.
[10] Teramachi M, Kiyotani T, Takimoto Y, Nakamura T, Shimizu Y. A new porous tracheal prosthesis sealed with collagen sponge. ASAIO J 1995;41:M306–10.
[11] Nakamura T, Teramachi M, Sekine T, Kawanami R, Fukuda S, Yoshitani M, et al. Artificial trachea and long term follow-up in carinal reconstruction in dogs. Int J Artif Organs 2000;23:718–24.
[12] 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.

[13] Yamashita M, Kanemaru S, Hirano S, Magrufov A, Tamaki H, Tamura Y, et al. Tracheal regeneration after partial resection: a tissue engineering approach. Laryngoscope 2007;117:497–502.

[14] Han J, Woytowich AE, Mandal AK, Hiebert LM. Heparanase upregulation in high glucose-treated endothelial cells is prevented by insulin and heparin. Exp Biol Med 2007;232: 927–34.

[15] Schultz GS, Wysocki A. Interactions between extracellular matrix and growth factors in wound healing. Wound Repair Regen 2009;17:153–62.

[16] O'Byrne KJ, Steward WP. Tumour angiogenesis: a novel therapeutic target in patients with malignant disease. Expert Opin Emerg Drugs 2001;6:155–74.

[17] Hirano S, Kishimoto Y, Suehiro A, Kanemaru S, Ito J. Regeneration of aged vocal fold: first human case treated with fibroblast growth factor. Laryngoscope 2008;118:2254–9.

[18] Parker NP, Bailey SS, Walner DL. Effects of basic fibroblast growth factor-2 and hyaluronic acid on tracheal wound healing. Laryngoscope 2009;119:734–9.

[19] Xie JL, Bian HN, Qi SH, Chen HD, Li HD, Xu YB, et al. Basic fibroblast growth factor (bFGF) alleviates the scar of the rabbit ear model in wound healing. Wound Repair Regen 2008;16:576–81.

[20] Takemoto S, Morimoto N, Kimura Y, Taira T, Kitagawa T, Tomihata K, et al. Preparation of collagen/gelatin sponge scaffold for sustained release of bFGF. Tissue Eng Part A 2008;14:1629–38.

The Laryngoscope

Chronic Vocal Fold Scar Restoration With Hepatocyte Growth Factor Hydrogel

Yo Kishimoto, MD; Shigeru Hirano, MD, PhD; Yoshiharu Kitani, MD; Atsushi Suehiro, MD; Hiroo Umeda, MD, PhD; Ichiro Tateya, MD, PhD; Shin-ichi Kanemaru, MD, PhD; Yasuhiko Tabata, PhD, DMedSci, DPharm; Juichi Ito, MD, PhD

Objectives/Hypothesis: Therapeutic challenges exist in the management of vocal fold scarring. We have previously demonstrated the therapeutic potential of hepatocyte growth factor (HGF) in the management of acute phase vocal fold scarring using a novel hydrogel-based HGF drug delivery system (DDS). However, the effect of HGF on matured vocal fold scarring remains unclear. The current study aims to investigate the effect of HGF-DDS on chronic vocal fold scarring using a canine model.

Study Design: Animal model.

Methods: Vocal folds from eight beagles were unilaterally scarred by stripping the entire layer of the lamina propria; contralateral vocal folds were kept intact as normal controls. Six months after the procedures, hydrogels (0.5 mL) containing 1 μg of HGF were injected into the scarred vocal folds of four dogs (HGF-treated group). Hydrogels containing saline solution were injected into the other four dogs (sham group). Histological and vibratory examinations on excised larynges were completed for each group 9 months after the initial surgery.

Results: Experiments conducted on excised larynges demonstrated significantly better vibrations in the HGF-treated group in terms of mucosal wave amplitude. Although phonation threshold pressure was significantly lower in the HGF-treated group compared with the sham group, no significant differences were observed in the normalized glottal gap between HGF-treated and sham groups. Histological examinations of the HGF-treated vocal folds showed reduced collagen deposition and less tissue contraction with favorable restoration of hyaluronic acid.

Conclusions: Results suggest that administration of HGF may have therapeutic potential in the treatment of chronic vocal fold scarring.

Key Words: Chronic vocal fold scarring, drug delivery system, hepatocyte growth factor.

***Laryngoscope*, 120:108–113, 2010**

INTRODUCTION

There continue to be therapeutic challenges in the management of vocal fold scarring.[1] Vocal fold scarring occurs following injury, inflammation, or phonosurgery and disrupts the layered structure of the lamina propria altering the biomechanical properties of the vocal fold. Vocal fold scarring often causes glottal insufficiency and severe intractable dysphonia.

Previous histologic studies[2–5] on vocal fold scarring have revealed changes in the organization and distribution of extracellular matrix components (ECM), such as dense and/or disorganized type I collagen deposition, decreased elastin and decorin, increased fibronectin, and occasional decreases in hyaluronic acid (HA). These results confirmed the aberrant synthetic phenotype of vocal fold scar fibroblasts.[6] Given that these histological changes stiffen the properties of the vocal fold, phenotypic changes of vocal fold fibroblasts and a correction of the distribution of ECM components is needed to restore the vocal fold after scarring.

Hepatocyte growth factor (HGF) is a multifunctional polypeptide that plays a significant role in embryogenesis, angiogenesis, organ regeneration, and wound healing.[7] HGF has strong antifibrotic potency and has been shown to contribute to the prevention or complete resolution of fibrosis in the liver, kidney, and lung in animal models.[7] Another study has shown the therapeutic potential of HGF in the management of vocal fold scarring by demonstrating that HGF can increase HA production and decrease collagen production in vocal fold fibroblasts.[8]

From the Department of Otolaryngology–Head and Neck Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan (Y.KISHIMOTO, S.H., Y.KITANI, H.U., I.T., J.I.); the Department of Otolaryngology, Vanderbilt University Bill Wilkerson Center for Otolaryngology and Communication Sciences, Nashville, Tennessee, U.S.A. (A.S.); the Department of Otolaryngology, Head and Neck Surgery, Kitano Hospital, Tazuke Kofukai Medical Research Institute, Osaka, Japan (S.-I.K.); and the Department of Biomaterials, Field of Tissue Engineering, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan (Y.T.).

Editor's Note: This Manuscript was accepted for publication June 18, 2009.

Send correspondence to Shigeru Hirano, Department of Otolaryngology–Head and Neck Surgery, Kyoto University Graduate School of Medicine, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan. E-mail: hirano@ent.kuhp.kyoto-u.ac.jp

DOI: 10.1002/lary.20642

We have previously shown the therapeutic potential of HGF in the management of vocal fold injury at acute phase.[9–11] Although those studies have shown the therapeutic potential of HGF, incomplete restoration of scarred tissue and individual variability of these effects were also reported.[11,12] These effects were attributed to insufficient retention time of HGF in the injected site, as the biological activity of HGF may be limited due to rapid dispersal by diffusion. To overcome this limitation and enhance the effect of HGF, we have developed a novel drug delivery system (DDS) for HGF using a gelatin hydrogel.[12] The previous study, however, revealed only the inhibiting effect on scar formation at the acute phase, and the effect of HGF on matured, chronic vocal fold scarring remains unclear. Given the clinical use of HGF it is important to determine if it has a restorative remodeling effect on chronic vocal fold scarring. The current study aims to investigate the effect of HGF-DDS on chronic vocal fold scarring using a canine model.

MATERIALS AND METHODS

Animals

Eight beagles weighing 10 to 17 kg were used in this study. All experimental protocols were approved by the Animal Committee of the Graduate School of Medicine, Kyoto University. Animal care was provided under the supervision of the Institute of Laboratory Animals of the Graduate School of Medicine, Kyoto University.

Preparation of HGF Hydrogel

Biodegradable hydrogels were developed by the Department of Biomaterials, Field of Tissue Engineering, Institute for Frontier Medical Sciences, Kyoto University.[13,14] The hydrogel was constituted by chemically cross-linking acidic gelatin with glutaraldehyde. A 50-mL quantity of acidic gelatin aqueous solution (5% w/w) was mixed with 50 μL of glutaraldehyde aqueous solution (25% w/w) to give a final concentration of 6.25 mM. The water content of the hydrogel was 94.8%. A solution of 1 μg of HGF (Human recombinant HGF; PeproTech Inc., Rocky Hill, NJ) in 20 μL of phosphate buffered saline (PBS) was dripped onto the gelatin hydrogels and left overnight at 4°C to create the HGF hydrogel.

Surgical Procedure

The surgical procedures that were used for generating the vocal fold injury models had been established in previous studies.[11,12] All animals were sedated under general anesthesia with intramuscular injections of ketamine hydrochloride (15 mg/kg) and xylazine hydrochloride (6 mg/kg). The glottis was visualized using a direct laryngoscope, and the vocal folds were unilaterally scarred by stripping the entire layer of the lamina propria down to the muscle. The contralateral vocal folds were kept intact as normal controls. The sides for scarring were randomly selected.

After stripping the vocal fold lamina propria of test animals, vocal fold scars were allowed to mature for 6 months. This period of vocal fold scar maturation was based on data from Rousseau et al., who proposed that it takes 6 months for vocal fold scarring to mature in canine and rat models.[3,4] Six months after the procedure, 0.5 mL of hydrogel solution containing 1 μg of HGF was injected into the scarred vocal folds of four dogs (HGF-treated group) using a transoral intracordal injector, and 0.5 mL of hydrogel solution containing 1 μg of PBS was injected into the scarred vocal folds of the four dogs in the sham group. Because HGF was expected to act on the fibroblast in the lamina propria, hydrogel was carefully injected into the subepithelial layer of the vocal fold. To enhance the effect of injection, the injection was performed twice at an interval of 1 month. It is reported that HGF acts on some kinds of cells in an autocrine manner,[15–17] and vocal fold fibroblasts may be similar to those cells. Thus, the effect of HGF might continue for some time after the administration and release period. For this reason, we set the interval to 1 month, which is longer than the 2-week release period.

All animals were euthanized 9 months after the surgery by intracardiac injection of Nembutal. The larynges were harvested and used for vibratory examinations then subjected to histological examination.

Setup for Vibratory Examination of Excised Larynges

Vocal fold vibration was examined with an excised larynx setup developed in previous studies.[10,12] For better visualization of the vocal folds supraglottic structures, including the epiglottis, false vocal folds and aryepiglottic folds were removed after resection of the superior portion of the thyroid cartilage. The arytenoid cartilages were sutured together, and an arytenoid adduction procedure was bilaterally performed using a 3-0 Prolene suture to close the glottis. The larynx was mounted on a table and an intubation tube was inserted into the trachea and tightly clamped. Air was pumped through the tube to generate vocal fold vibrations. During the vibratory examination, saline was dripped onto the vocal folds to prevent dehydration. A pressure sensor (PG-100; Nidec Copal Electronics Corp., Tokyo, Japan) was inserted into the tube to monitor subglottic pressure, and a high-speed digital imaging system (MEMRECAMci; NAC Image Technology, Osaka, Japan) was used to record vocal fold vibrations from the superior view. The camera was mounted 50 cm above the larynx, and the image was displayed on a monitor. The images were recorded at a frame rate of 1,000 frames per second, which is the maximum rate to give an acceptable resolution level in our equipment, and the images were then scanned into a computer.

As an indirect measurement, we used phonation threshold pressure (PTP) to evaluate the mucosal vibration. PTP, which is regulated by factors such as vocal fold thickness, property, and glottal width, is defined as the minimum pressure required to initiate phonation.[18,19] Further, the amplitude of the mucosal wave and glottal gap were measured using image analysis software (Scion Image beta4; Scion Corp., Frederick, MD). The distance (d1) from the midline of the glottis to the free edge of the vocal fold was measured at the anteroposterior middle portion of the vocal fold during the closed phase. Closed phase was recognized by the motion of the upper and lower lips of the vocal folds. The same distance (d2) was measured at the maximum open phase. The mucosal wave amplitude was defined by subtracting d1 from d2 and the amplitude ratio was derived by dividing the amplitude in the HGF-treated side by the amplitude in the normal side. The following formula was used: amplitude ratio (AR) = (d2−d1 in the HGF-treated side)/(d2−d1 in the normal side). The glottal gap was examined from the images during the closed phase. The length (L) from the anterior commissure to the vocal process and the glottal area (a) were measured, and the glottal area was normalized by dividing it by L^2. The following formula was used: normalized glottal gap (NGG) = $a/L^2 \times 100$ unit (u).

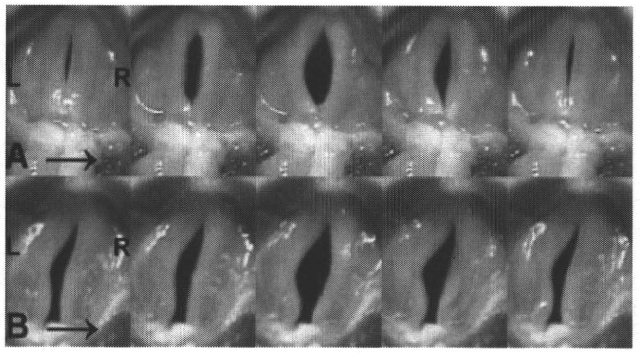

Fig. 1. Vibratory patterns experimentally generated from treated vocal folds of excised larynges. In both cases the left vocal fold was scarred. The hepatocyte growth factor-treated vocal fold showed almost normal mucosal vibration (A), however the sham-treated vocal fold was bowed and mucosal vibration was limited (B).

Histological Examination

Collagen, elastin, and HA in the lamina propria of each vocal fold were examined using light microscopy. The thickness of the lamina propria was also assessed to determine the degree of scar contraction. The thickness of the lamina propria was determined by measuring the distance from the free edge of the vocal fold down to the thyroarytenoid muscle and normalized by dividing the distance on the treated side (t1) by that of the normal side (t2). The following formula was used: normalized thickness of lamina propria (NTLP) = t1/t2.

Immediately following the vibratory examinations, the larynges were fixed in 10% formaldehyde for later tissue examination. Larynges were subsequently embedded in paraffin, and 5-μm-thick serial sections were prepared in the coronal plane from the anteroposterior middle portion of the vocal folds.

Elastica van Gieson staining was performed to identify collagen and elastin. Alcian blue staining was used to identify HA. A hyaluronidase digestion technique was used to detect HA. Images were captured with a BIOREVO BZ-9000 microscope (Keyence Corp., Osaka, Japan).

These assessments were performed in a blinded fashion, in which the examiners were not informed which slide belonged to each group.

Statistical Analysis

An unpaired t test was used to ascertain differences in PTP, AR, NGG, and NTLP between treatment groups. A P value $< .05$ was considered statistically significant.

RESULTS

Vibratory Examinations

The experiments on excised larynges showed better mucosal vibration in the HGF-treated group, as compared with the sham group. Figure 1 shows representative cases in the HGF-treated group (Fig. 1A) and in the sham group (Fig. 1B). Injured vocal folds were bowed and the mucosal vibration was limited in the sham group; however, their vibration was comparable to the uninjured side in the HGF-treated group.

An unpaired t test revealed significantly lower PTP in the HGF-treated group, compared with the sham group (Fig. 2A, $P = .015$). Although no significant differences were observed for NGG between the two groups (Fig. 2B), AR was significantly higher in the HGF-treated group compared to the sham group (Fig. 2C, $P = .012$).

Histological Examinations

Histological examinations revealed better restoration and less tissue contraction in the HGF-treated vocal fold compared with the sham-treated vocal fold.

Disorganized collagen deposition was found to be minimal in the HGF-treated vocal fold (Fig. 3A, 3B), whereas there was excessive collagen deposition in the sham-treated vocal fold (Fig. 4A, 4B). Elastin and HA in the HGF-treated vocal fold appeared to be well

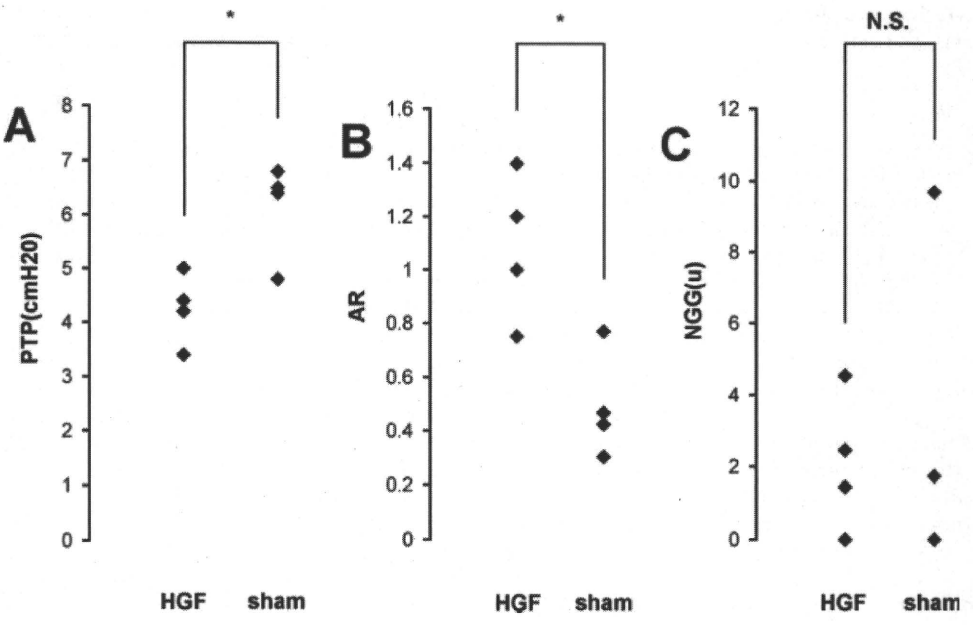

Fig. 2. Results of vibratory examinations. The hepatocyte growth factor (HGF)-treated group demonstrated significantly lower phonation threshold pressure (PTP) (A) and higher amplitude ratio (AR) (B) compared with the sham-treated group. No differences were observed for normalized glottal gap (NGG) between the two groups (C). * $P < .05$.

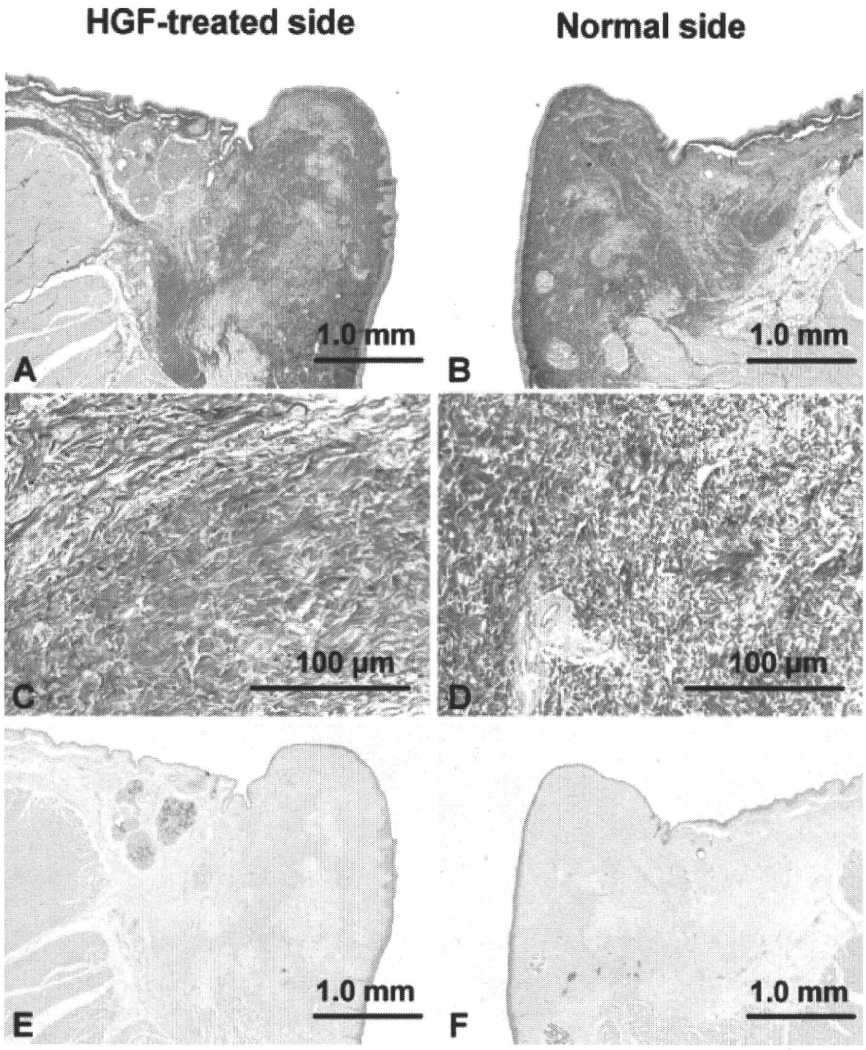

Fig. 3. Histologic findings in the hepatocyte growth factor (HGF)-treated group. (A–D) Elastica van Gieson stain. (E–F) Alcian blue stain. Tissue contraction and collagen deposition were found to be minimal (A, B), and elastin (C, D) and hyaluronic acid (E, F) were favorably restored.

organized, similar to that of the unscarred contralateral side (Fig. 3C–3F), whereas both were decreased in the sham-treated vocal fold (Fig. 4C–4F). There were no remarkable findings in terms of the underlying muscle.

NTLP was close to normal in the HGF-treated vocal folds, whereas NTLP was significantly thinner in the sham group as compared with the HGF-treated group (Fig. 5, $P = .03$).

DISCUSSION

The restoration of normal vocal fold properties is essential to the treatment of vocal fold scarring. With the advancement of phonosurgery, most voice disorders have been overcome, and many therapeutic strategies, including medialization thyroplasty, fat/collagen injection, and scar dissection have been tried in an attempt to restore normal properties to scarred vocal folds.[20] Medialization thyroplasty and fat/collagen injection result in augmentation effects that improve glottal insufficiency and facilitate entrainment of vocal fold vibrations. However, the restoration of normal vocal fold properties is not achieved by these treatments. The effect of scar dissection depends on the individual's healing ability, and stable outcomes with this approach cannot be achieved. Thus, there is no optimal strategy for the treatment of vocal fold scarring to date, and development of a new regenerative pathway is needed.

In tissue engineering, regeneration of tissues or organs can be achieved by the combination of scaffold, cells, and regulatory factors under appropriate conditions. Applying this concept, we have focused on two kinds of therapeutic strategies—cell therapy[21] and growth factor therapy[8–12]—for the treatment of vocal fold scarring. As a cell source for cell therapy, we have shown the therapeutic potential of autologous mesenchymal stem cells (MSCs).[21] We have previously injected MSCs into injured vocal folds at an acute phase in a canine model, and histologic examinations revealed improved healing after 2 months. Furthermore, we have shown the efficacy of HGF as a candidate for growth factor therapy in the management of vocal fold scarring by demonstrating its ability to control ECM production in vocal fold fibroblasts. As described before, HGF has strong antifibrotic potency and has been shown to contribute to the prevention or complete resolution of

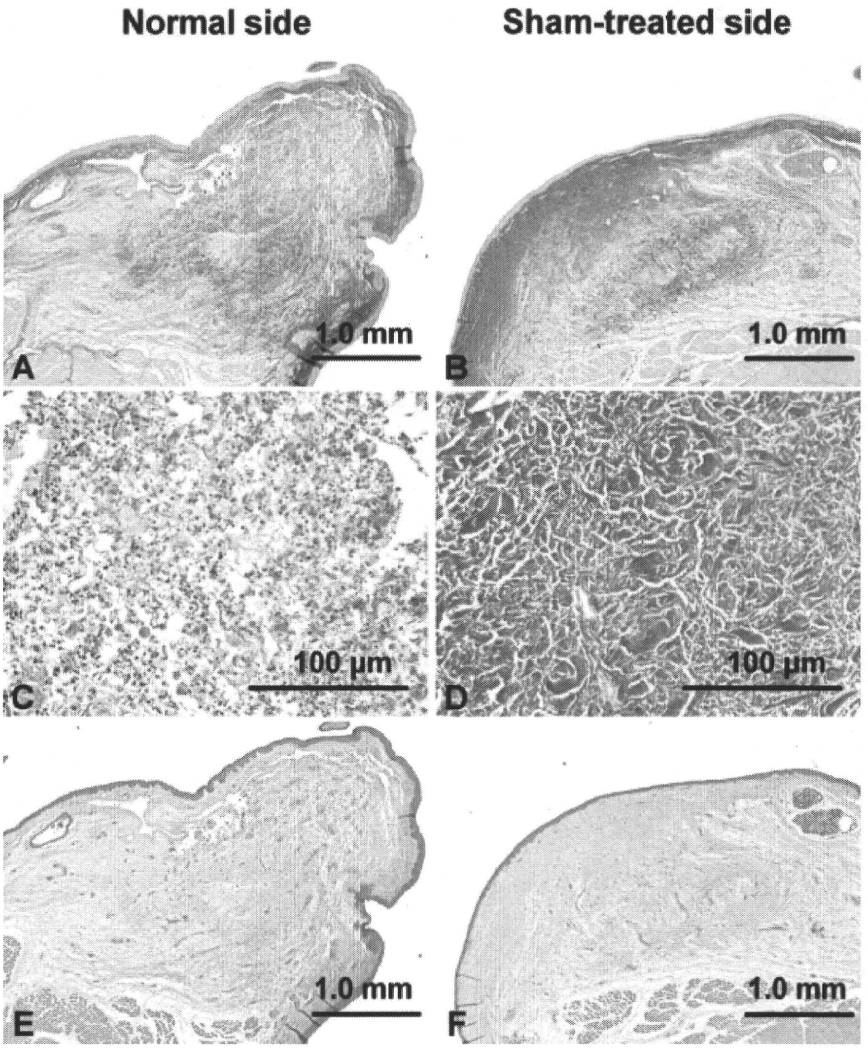

Fig. 4. Histologic findings in the sham-treated group. (A–D) Elastica van Gieson stain. (E–F) Alcian blue stain. Severe tissue contraction and excessive collagen deposition were observed in sham-treated vocal folds (A, B). Elastin (C, D) and hyaluronic acid (E, F) were decreased in the superior portion of the treated vocal fold.

fibrosis in some organs.[7] Hirano et al. reported the effects of HGF for the treatment of acute vocal fold injury using canine[11] and rabbit[10] models. In these studies, HGF was injected into injured vocal folds, and histological examination revealed reduced collagen deposition and decreased tissue contraction of the lamina propria in HGF-injected vocal folds as compared with saline-injected controls. However, these previous studies revealed only the inhibiting effect on scar formation at the acute phase of wound healing, and it is not clear whether these approaches have a restorative effect on the aberrant synthetic phenotype of vocal fold fibroblasts. In the current study we have investigated the effect of HGF administration on chronic matured vocal fold scarring.

A biodegradable hydrogel developed to enhance the in vivo regenerative effects of growth factors, such as HGF, basic fibroblast growth factor, platelet-derived growth factor, and epidermal growth factor, has been shown to be successful in the controlled release of biologically active growth factors in other parts of body.[13,14,22–24] In this system, HGF was embedded in gelatin hydrogel and gradually released in a continuous fashion over a 2-week period in vivo.

This study represents the first investigation of HGF for the treatment of matured, chronic vocal fold scarring in vivo. The vibratory experiments in the present study showed significant improvement of mucosal vibration in terms of PTP and AR in the HGF-treated group compared with the sham group. As mentioned above, PTP is regulated by the vocal fold property and glottal gap. Improvement in PTP without smaller NGG indicates that administration of HGF restored the scarred vocal fold in terms of stiffness and tissue contraction. Histological examination also showed positive restorative effects with the administration of HGF, including reduced collagen deposition, less tissue contraction, and improved restoration of elastin and HA. These results suggest that HGF-DDS has restorative remodeling effects on chronic vocal fold scarring; however, there was still individual variability and complete restoration could not be achieved. Particularly, a possible reason for incomplete improvement of glottal gap may be insufficient volume obtained in the treated vocal folds. Here may be some limitations in growth factor therapy, which warrant a combined use of cells and/or appropriate scaffoldings to obtain adequate tissue volume and function.

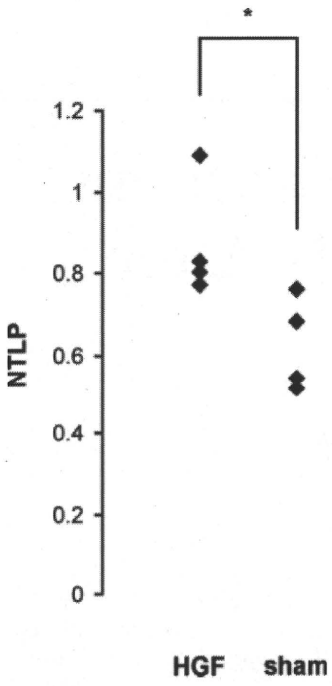

Fig. 5. Normalized thickness of lamina propria (NTLP) was significantly thinner in the sham-treated group than in the hepatocyte growth factor (HGF)-treated group. *$P < .05$.

CONCLUSION

The present study demonstrated that the HGF-DDS significantly improved the vibratory properties of matured, chronic vocal fold scarring in a canine model. HGF-DDS reduced excessive collagen deposition and tissue contraction with favorable restoration of elastin and HA. Results suggest that administration of HGF may have therapeutic potential in the treatment of chronic vocal fold scarring.

BIBLIOGRAPHY

1. Hirano S. Current treatment of vocal fold scarring. *Curr Opin Otolaryngol Head Neck Surg* 2005;13:143–147.
2. Hirano S, Minamiguchi S, Yamashita M, Ohno T, Kanemaru SI, Kitamura M. Histologic characterization of human scarred vocal folds. *J Voice* 2009;23:399–407.
3. Rousseau B, Hirano S, Scheidt TD, et al. Characterization of vocal fold scarring in a canine model. *Laryngoscope* 2003;113:620–627.
4. Rousseau B, Hirano S, Chan RW, et al. Characterization of chronic vocal fold scarring in a rabbit model. *J Voice* 2004;18:116–124.
5. Tateya T, Tateya I, Sohn JH, Bless DM. Histologic characterization of rat vocal fold scarring. *Ann Otol Rhinol Laryngol* 2005;114:183–191.
6. Krishna P, Rosen CA, Branski RC, Wells A, Hebda PA. Primed fibroblasts and exogenous decorin: potential treatments for subacute vocal fold scar. *Otolaryngol Head Neck Surg* 2006;135:937–945.
7. Matsumoto K, Nakamura T. Hepatocyte growth factor (HGF) as a tissue organizer for organogenesis and regeneration. *Biochem Biophys Res Commun* 1997;239:639–644.
8. Hirano S, Bless D, Heisey D, Ford C. Roles of hepatocyte growth factor and transforming growth factor beta1 in production of extracellular matrix by canine vocal fold fibroblasts. *Laryngoscope* 2003;113:144–148.
9. Hirano S, Bless DM, Heisey D, Ford CN. Effect of growth factors on hyaluronan production by canine vocal fold fibroblasts. *Ann Otol Rhinol Laryngol* 2003;112:617–624.
10. Hirano S, Bless DM, Rousseau B, et al. Prevention of vocal fold scarring by topical injection of hepatocyte growth factor in a rabbit model. *Laryngoscope* 2004;114:548–556.
11. Hirano S, Bless DM, Nagai H, et al. Growth factor therapy for vocal fold scarring in a canine model. *Ann Otol Rhinol Laryngol* 2004;113:777–785.
12. Ohno T, Hirano S, Kanemaru S, et al. Drug delivery system of hepatocyte growth factor for the treatment of vocal fold scarring in a canine model. *Ann Otol Rhinol Laryngol* 2007;116:762–769.
13. Ozeki M, Ishii T, Hirano Y, Tabata Y. Controlled release of hepatocyte growth factor from gelatin hydrogels based on hydrogel degradation. *J Drug Target* 2001;9:461–471.
14. Ikada Y, Tabata Y. Protein release from gelatin matrices. *Adv Drug Deliv Rev* 1998;31:287–301.
15. Wordinger RJ, Clark AF, Agarwal R, et al. Cultured human trabecular meshwork cells express functional growth factor receptors. *Invest Ophthalmol Vis Sci* 1998;39:1575–1589.
16. Sheehan SM, Tatsumi R, Temm-Grove CJ, Allen RE. HGF is an autocrine growth factor for skeletal muscle satellite cells in vitro. *Muscle Nerve* 2000;23:239–245.
17. Yang XM, Toma JG, Bamji SX, et al. Autocrine hepatocyte growth factor provides a local mechanism for promoting axonal growth. *J Neurosci* 1998;18:8369–8381.
18. Titze IR. The physics of small-amplitude oscillation of the vocal folds. *J Acoust Soc Am* 1988;83:1536–1552.
19. Titze IR. Phonation threshold pressure: a missing link in glottal aerodynamics. *J Acoust Soc Am* 1992;91: 2926–2935.
20. Dailey SH, Ford CN. Surgical management of sulcus vocalis and vocal fold scarring. *Otolaryngol Clin North Am* 2006; 39:23–42.
21. Kanemaru S, Nakamura T, Omori K, et al. Regeneration of the vocal fold using autologous mesenchymal stem cells. *Ann Otol Rhinol Laryngol* 2003;112:915–920.
22. Haraguchi T, Okada K, Tabata Y, Maniwa Y, Hayashi Y, Okita Y. Controlled release of basic fibroblast growth factor from gelatin hydrogel sheet improves structural and physiological properties of vein graft in rat. *Arterioscler Thromb Vasc Biol* 2007;27:548–555.
23. Hokugo A, Sawada Y, Hokugo R, et al. Controlled release of platelet growth factors enhances bone regeneration at rabbit calvaria. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007;104:44–48.
24. Hori K, Sotozono C, Hamuro J, et al. Controlled-release of epidermal growth factor from cationized gelatin hydrogel enhances corneal epithelial wound healing. *J Control Release* 2007;118:169–176.

The Laryngoscope

Temporal Changes in Vocal Functions of Human Scarred Vocal Folds After Cordectomy

Yo Kishimoto, MD; Shigeru Hirano, MD, PhD; Ichiro Tateya, MD, PhD; Shin-Ichi Kanemaru, MD, PhD; Juichi Ito, MD, PhD

Objectives/Hypothesis: The maturation process of scarred vocal folds has previously been investigated using animal models. However, in human models the features of scarred vocal folds have rarely been described, and the process by which the scar changes with time is not well known. The present study aimed to investigate the maturation process of human vocal folds scarred by cordectomy in terms of vibratory and aerodynamic functions.

Study Design: Prospective case series.

Methods: Eight patients with early glottic carcinoma and two patients with leukoplakia of the vocal fold underwent endoscopic cordectomy at Kyoto University Hospital between 2006 and 2008. The temporal changes in their vocal functions were evaluated using acoustic and aerodynamic analyses and videostroboscopic examination.

Results: Normalized mucosal wave amplitude, mean flow rate, and the amplitude perturbation quotient appear to stabilize about 6 months after the procedure. Although there were individual variations in the changes in normalized glottal gap and maximum phonation time, it appears to take at least 6 months to reach plateau. The other parameters—pitch perturbation quotient and noise to harmonic ratio—varied by individual, and thus it was difficult to identify commonalities in the healing process.

Conclusions: Some individual variation was observed in the temporal changes of vocal function of scarred vocal folds after cordectomy. However, in terms of vibratory and aerodynamic functions, this study suggests that it takes at least 6 months for maturation of vocal fold scarring.

Key Words: vocal function, human, scarred vocal fold, maturation.

Level of Evidence: 4

***Laryngoscope*, 120:1597–1601, 2010**

INTRODUCTION

Vocal fold scarring occurs after injury or inflammation of the vocal fold mucosa. It impairs mucosal vibration and results in severe, intractable dysphonia. Given that voice is one of the most important tools for communication, dysphonia might lead to psychological and social distress. Although many voice disorders have been overcome by advances in voice therapy and/or phonosurgery, to date there is no optimized therapeutic strategy for vocal fold scarring.[1]

To develop a therapeutic strategy for the management of vocal fold scarring, it is necessary to understand the process of vocal fold scar maturation. Previously, animal models have been used to characterize the features of scarred vocal folds.[2–4] In these studies, histological analysis of scarred vocal folds has revealed changes in the organization and distribution of extracellular matrix components, including dense and/or disorganized type I collagen deposition, decreased elastin and decorin, increased fibronectin, and occasional decreases in hyaluronic acid.[2–4] Similar changes have also been reported for the scarred vocal folds of humans.[5]

Rousseau et al. have proposed that it takes 6 months for vocal fold scarring to mature in canine and rabbit models.[2,3] However, owing to the difficulty in performing histological evaluations on human vocal folds, the features of scarred human vocal folds have rarely been characterized. Similarly, how the scar changes over time is not well known. To properly evaluate and diagnose scarred lesions and to determine the proper

From the Department of Surgery, Division of Otolaryngology–Head and Neck Surgery, University of Wisconsin School of Medicine and Public Health (Y.K.), Madison, Wisconsin, U.S.A.; the Department of Otolaryngology–Head and Neck Surgery, Graduate School of Medicine, Kyoto University (Y.K., S.H., I.T., J.I.) Kyoto, Japan; and the Department of Otolaryngology, Head and Neck Surgery, Kitano Hospital, Tazuke Kofukai Medical Research Institute (S.K.), Osaka, Japan.

Editor's Note: This Manuscript was accepted for publication March 15, 2010.

This study was supported in part by a grant from Takeda Science Foundation. The authors have no other funding, financial relationships, or conflicts of interest to disclose.

Send correspondence to Shigeru Hirano, MD, Department of Otolaryngology–Head and Neck Surgery, Graduate School of Medicine, Kyoto University, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan. E-mail: hirano@ent.kuhp.kyoto-u.ac.jp

DOI: 10.1002/lary.21016

TABLE I.
Clinical Information About the Patients and Surgical Procedures.

Case number	Sex	Age (years)	Side	Cordectomy type	Diagnosis
1	M	55	Rt.	I	Carcinoma
2	M	69	Bil.	I	Carcinoma
3	M	65	Bil.	I	Leukoplakia
4	M	70	Rt.	II	Carcinoma
5	M	64	Rt.	I	Carcinoma
6	F	47	Bil.	I	Carcinoma
7	M	74	Lt.	I	Carcinoma
8	F	53	Lt.	I	Leukoplakia
9	M	56	Rt.	III	Carcinoma
10	M	69	Lt.	II	Carcinoma

M: Male, F: female, Rt.: Right, Lt.: Left, Bil.: Bilateral.

therapeutic strategy, it is essential to know how the vocal fold scar changes and matures over time.

Because human vocal function can be followed temporally, we have focused on the functional rather than histological changes in the process of vocal fold scarring. To know the changes in vocal function during the scar maturation would help us to understand its condition. In the current study, we investigated this maturation process by examining the temporal changes in vibratory, acoustic, and aerodynamic properties of scarred vocal folds in postcordectomy patients.

MATERIALS AND METHODS

Patients

Clinical information about the patients and surgical procedures is summarized in Table I. Ten patients (eight men and two women), eight with early glottic carcinoma and two with leukoplakia of the vocal fold, underwent endoscopic cordectomy from 2006 to 2008 at Kyoto University Hospital. Their ages ranged from 47 to 74 years (average, 62 years). Seven patients underwent type I cordectomy, two patients underwent type II cordectomy, and one patient underwent type III cordectomy. Seven cases were treated with unilateral resections, whereas the others received bilateral resections.

Assessments

Two trained laryngologists made blind measurements. Assessment consisted of stroboscopic, acoustic, and aerodynamic examinations. Voice and stroboscopic samples were recorded three times at normal pitch and loudness in each examination.

Stroboscopic examinations were performed with a Digital Video System Model 9295 (Kay PENTAX, Lincoln Park, NJ) to assess temporal changes in the mucosal wave and glottic closure. The amplitude of the mucosal wave and glottal gap was examined using image analysis software (Scion Image Beta3b; Scion Corp., Frederick, MA). The distance (d1) from the midline of the glottis to the free edge of the vocal fold was measured at the anteroposterior middle portion of the vocal fold during the closed phase, and then the same distance (d2) was measured at the maximum open phase. Mucosal wave amplitude was normalized by the distance (L) from the anterior commissure to the vocal process. The normalized mucosal wave amplitude (NMWA) was calculated from the formula NMWA = (d2 − d1)/L. This measurement was done on the treated side of the vocal fold. Glottal gap was examined in the images at closed phase. Glottal area (a) was measured, and the normalized glottal gap (NGG) was calculated as $NGG = a/L^2$.

Aerodynamic examinations included maximum phonation time (MPT) and mean flow rate (MFR). For acoustic examinations, Computerized Speech Lab software (Kay PENTAX) was used to evaluate pitch perturbation quotient (PPQ), amplitude perturbation quotient (APQ) and noise to harmonic ratio (NHR).

RESULTS

Vibratory Examinations

Figure 1 shows the temporal changes of NMWA and NGG. NMWA showed gradual improvement in all cases, and it appears to stabilize about 6 months after the procedure. Although there were individual variations in the changes of NGG, it appears to take at least 6 months to reach plateau.

Aerodynamic and Acoustic Examinations

Although the changes in MPT differed by individual, the majority of cases had stabilized by 6 months after surgery (Fig. 2A). Similarly, MFR also appeared to become stable 6 months postprocedure (Fig. 2B).

In some cases (case 2 at 2 months postsurgery and case 10) the voice was too harsh and rough for acoustic analysis to be performed. Although APQ showed gradual improvement and stabilized after about 6 months (Fig. 2D), the individual variability within the other parameters—PPQ and NHR—made it difficult to draw any generalized conclusions about the healing process (Fig. 2C, 2E).

Representative Case

Post-bilateral type I cordectomy scar (case 2). A 69-year-old man with laryngeal carcinoma in both vocal folds underwent bilateral type I cordectomy. Two months after surgery, no mucosal waves were seen in either of the vocal folds, and incomplete glottal closure was observed (Fig. 3A). Four months after surgery, the

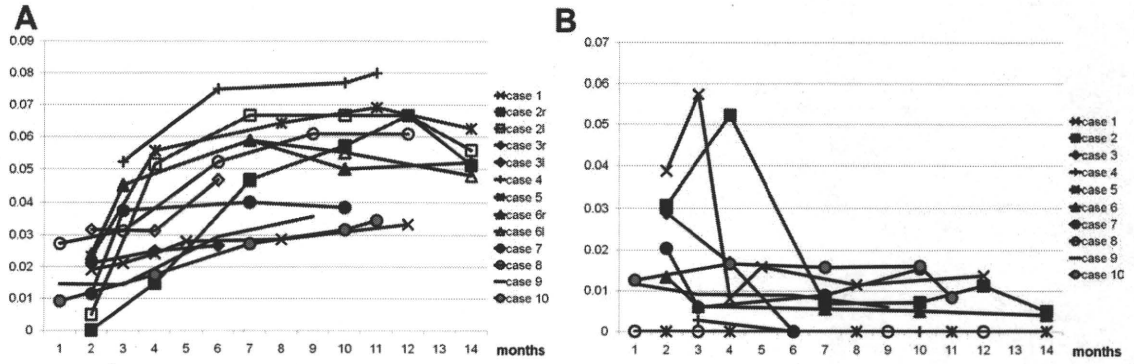

Fig. 1. Temporal changes in normalized mucosal wave amplitude (NMWA) and normalized glottal gap (NGG). Although there were individual variations in the changes of NGG (B), NMWA (A) and NGG appear to stabilize about 6 months after the procedure. r = right vocal fold; l = left vocal fold.

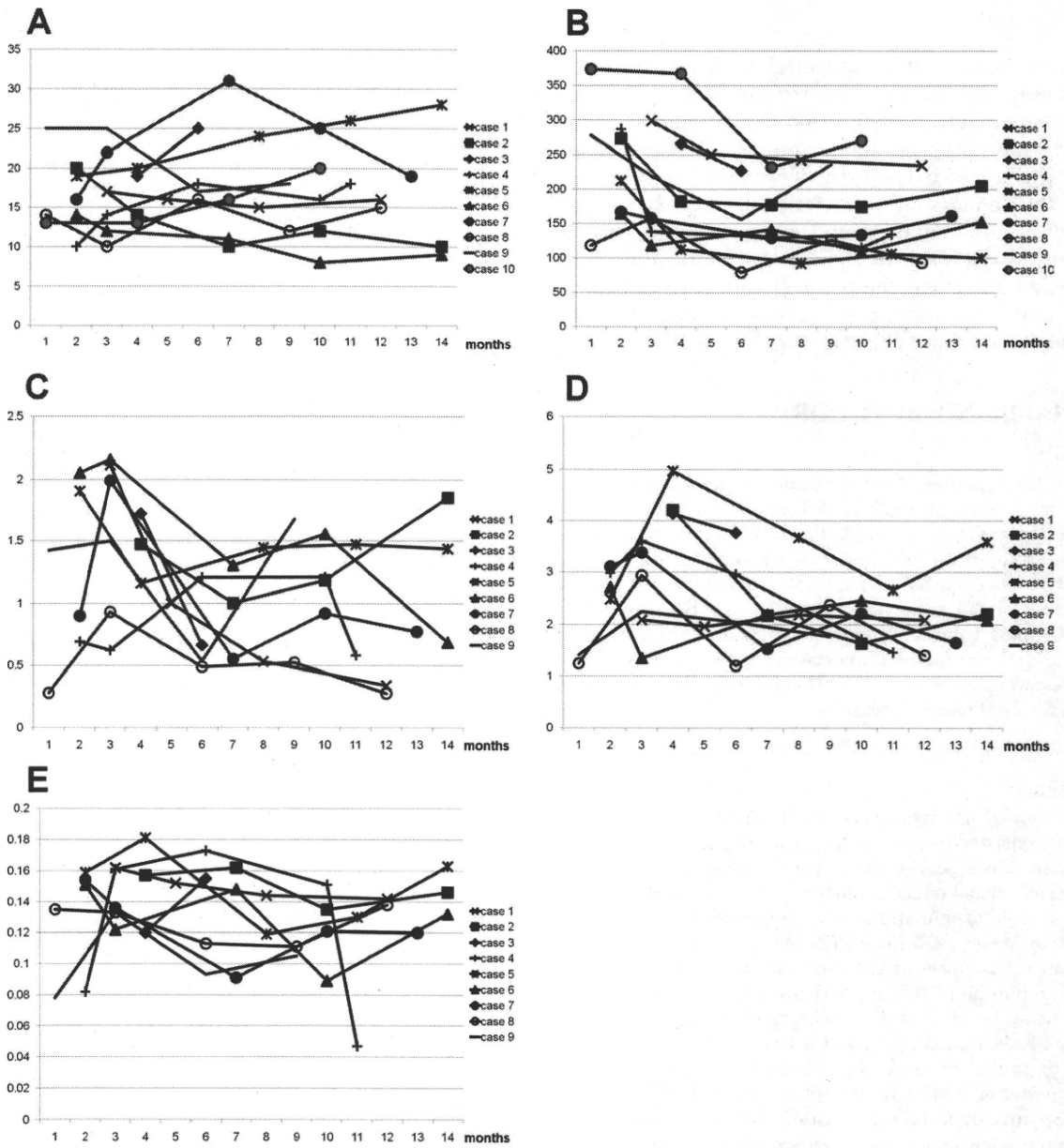

Fig. 2. Temporal changes in aerodynamic and acoustic measurements. (A) Maximum phonation time (MPT). (B) Mean flow rate (MFR). (C) Pitch perturbation quotient (PPQ). (D) Amplitude perturbation quotient (APQ). (E) Noise to harmonic ratio (NHR). The majority of cases had stabilized by 6 months after surgery in MPT and MFR. Though PPQ and NHR varied individually, APQ stabilized after about 6 months.

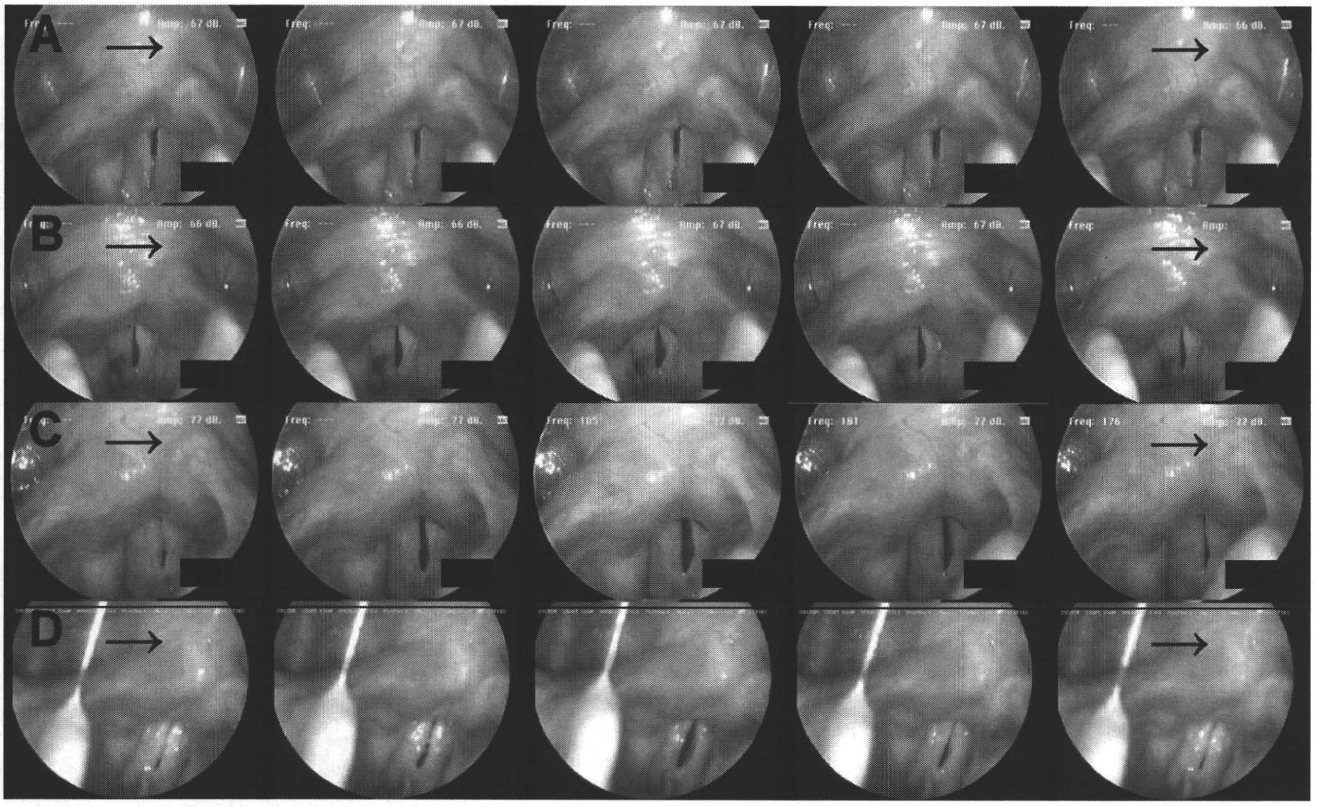

Fig. 3. Stroboscopic findings in case 2. No mucosal waves were seen in either vocal fold 2 months after surgery (A). Four months after surgery, the vocal folds showed some vibration; however, the vibration of the right vocal fold was still limited (B). Improved mucosal waves were observed 7 (C) and 14 (D) months after the procedure.

vocal folds showed some ability to vibrate; however, the vibration of the right vocal fold remained limited (Fig. 3B). Although an anterior glottic web was present, the glottal gap had become smaller and improved mucosal waves were observed 7 months after the procedure (Fig. 3C). These improvements continued 14 months after the procedure (Fig. 3D).

DISCUSSION

With recent developments in regenerative medicine, new therapeutic strategies are being established for previously intractable disorders of organs systems.[6,7] In the treatment of vocal fold scarring, various restorative approaches based on the principles of tissue engineering—cell therapy,[8,9] growth factor therapy,[10–13] and scaffolding therapy[14–16]—have been utilized in attempts to restore normal function. Although these approaches are not fully established, some restorative effects have been reported and show promise as the basis for a new unified therapeutic strategy. To measure the clinical potential of such new approaches, it is important to have a comprehensive understanding, including the functional aspects, of the maturation process of human vocal fold scarring.

Cordectomy, which is one of the main treatments for early glottic carcinoma and laryngeal precancerous lesion, is a common cause of iatrogenic vocal fold scarring. Cordectomy has been shown to have high cure rates for these conditions while enabling laryngeal preservation.[17] It has also attracted much attention as a minimally invasive therapeutic strategy because it offers several advantages over radiation therapy, including the elimination of the adverse effects of irradiation, shorter hospitalization times, and high cost effectiveness.[17]

Postcordectomy patients were enlisted for this study as it is possible to precisely identify the onset of the scarring process following surgery. The wound healing response consists of three successive but overlapping phases—inflammation, proliferation, and tissue remodeling. Wound healing has been well documented on cutaneous wounds, and some valuable data pertaining to vocal fold scarring also exists.[18,19] A previous study on acute vocal fold injury reported that the vocal fold wound healing process is analogous to wound repair in the skin during the inflammatory and proliferative phases, but differs during the remodeling phase.[20] Additionally, Bond et al. have reported that cutaneous wound scar redness fades at 7 months, and scar maturation occurs over the course of 1 year.[21,22] Consistent with this, Xu et al. have reported that vocal quality becomes steady 6 months after surgery in type III–IV cordectomy patients.[17] In the current study, vibratory, aerodynamic, and acoustic parameters required at least 6 months to stabilize, and full scar maturation is thought to occur over a period of 1 year. These results suggest that the

maturation process in vocal fold scarring is temporally similar to that of cutaneous scarring.

The presence of individual variation in vibratory and functional changes of the scarred vocal folds made it difficult to identify commonalities for some parameters. These variations are thought to primarily depend on the individual healing mechanisms. Moreover, most patients in this case series underwent type I cordectomy, which is the shallowest resection. The variations might stand out because the scarring effect of this procedure is thought to be minimal and does not obscure the individual variations in healing ability. Furthermore, MPT and acoustic parameters are affected by other factors, such as pulmonary function, and might not accurately depict the condition of the vocal fold.

This study has identified the temporal changes that occur in vibratory function during maturation of human vocal fold scarring. Although these temporal changes do not reflect the maturation process of vocal fold scarring directly, they would help our understanding of the process and complement other basic science work in providing benchmarks in clinical use. This is, however, a preliminary study owing to the small number of enlisted patients. Further study is necessary to fully understand the wound healing process that occurs following vocal fold injury.

CONCLUSION

Some individual variation was observed in the temporal changes of vocal function of scarred vocal folds after cordectomy. However, in terms of vibratory and aerodynamic functions, this study suggests that it takes at least 6 months for maturation of vocal fold scarring.

BIBLIOGRAPHY

1. Hirano S. Current treatment of vocal fold scarring. *Curr Opin Otolaryngol Head Neck Surg* 2005;13:143–147.
2. Rousseau B, Hirano S, Scheidt TD, et al. Characterization of vocal fold scarring in a canine model. *Laryngoscope* 2003;113:620–627.
3. Rousseau B, Hirano S, Chan RW, et al. Characterization of chronic vocal fold scarring in a rabbit model. *J Voice* 2004;18:116–124.
4. Tateya T, Tateya I, Sohn JH, Bless DM. Histologic characterization of rat vocal fold scarring. *Ann Otol Rhinol Laryngol* 2005;114:183–191.
5. Hirano S, Minamiguchi S, Yamashita M, Ohno T, Kanemaru SI, Kitamura M. Histologic characterization of human scarred vocal folds. *J Voice* 2009;23:399–407.
6. Laflamme MA, Murry CE. Regenerating the heart. *Nat Biotechnol* 2005;23:845–856.
7. Kimura Y, Tabata Y. Experimental tissue regeneration by DDS technology of bio-signaling molecules. *J Dermatol Sci* 2007;47:189–199.
8. Kanemaru S, Nakamura T, Omori K, et al. Regeneration of the vocal fold using autologous mesenchymal stem cells. *Ann Otol Rhinol Laryngol* 2003;112:915–920.
9. Chhetri DK, Head C, Revazova E, Hart S, Bhuta S, Berke GS. Lamina propria replacement therapy with cultured autologous fibroblasts for vocal fold scars. *Otolaryngol Head Neck Surg* 2004;131:864–870.
10. Hirano S, Bless DM, Nagai H, et al. Growth factor therapy for vocal fold scarring in a canine model. *Ann Otol Rhinol Laryngol* 2004;113:777–785.
11. Hirano S, Kishimoto Y, Suehiro A, Kanemaru S, Ito J. Regeneration of aged vocal fold: first human case treated with fibroblast growth factor. *Laryngoscope* 2008;118: 2254–2259.
12. Ohno T, Yoo MJ, Swanson ER, Hirano S, Ossoff RH, Rousseau B. Regeneration of aged rat vocal folds using hepatocyte growth factor therapy. *Laryngoscope* 2009;119: 1424–1430.
13. Kishimoto Y, Hirano S, Kitani Y, et al. Chronic vocal fold scar restoration with hepatocyte growth factor hydrogel. *Laryngoscope* 2010;120:108–113.
14. Kishimoto Y, Hirano S, Kojima T, Kanemaru S, Ito J. Implant of atelocollagen sheet for the treatment of vocal fold scarring and sulcus vocalis. *Ann Otol Rhinol Laryngol* 2009;118:613–620.
15. Duflo S, Thibeault SL, Li W, Shu XZ, Prestwich GD. Vocal fold tissue repair in vivo using a synthetic extracellular matrix. *Tissue Eng* 2006;12:2171–2180.
16. Xu CC, Chan RW. Pore architecture of a bovine acellular vocal fold scaffold. *Tissue Eng Part A* 2008;14:1893–1903.
17. Xu W, Han D, Hou L, Zhang L, Yu Z, Huang Z. Voice function following CO2 laser microsurgery for precancerous and early-stage glottic carcinoma. *Acta Otolaryngol* 2007; 127:637–641.
18. Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. *Nature* 2008;453:314–321.
19. Li J, Chen J, Kirsner R. Pathophysiology of acute wound healing. *Clin Dermatol* 2007;25:9–18.
20. Tateya T, Tateya I, Sohn JH, Bless DM. Histological study of acute vocal fold injury in a rat model. *Ann Otol Rhinol Laryngol* 2006;115:285–292.
21. Bond JS, Duncan JA, Sattar A, et al. Maturation of the human scar: an observational study. *Plast Reconstr Surg* 2008;121:1650–1658.
22. Bond JS, Duncan JA, Mason T, et al. Scar redness in humans: how long does it persist after incisional and excisional wounding? *Plast Reconstr Surg* 2008;121: 487–496.

<< Back to document

Glottal Reconstruction With a Tissue Engineering Technique Using Polypropylene Mesh: A Canine Experiment

Yamashita, Masaru; Kanemaru, Shin-ichi; Hirano, Shigeru; Umeda, Hiroo; Kitani, Yoshiharu; ###.
The Annals of Otology, Rhinology & Laryngology119.2 (Feb 2010): 110-7.

Abstract

OBJECTIVES: The larynx must be resected in some cases of cancer or stenosis, and various techniques are generally employed to fill the resulting defect. No ideal way, however, has been established to restore vocal function after this form of insult. The aim of this preliminary feasibility study in a canine model was to investigate the effectiveness of a polypropylene-based tissue engineering approach to repair a partial glottal defect. METHODS: Eight dogs were used in this study. A laryngeal defect involving resection of the left vocal fold was created through a thyroid cartilage window. A scaffold made of polypropylene and collagen was preclotted and wrapped with autologous fascia lata, inserted through the window, and sutured to the laryngeal defect in 5 dogs. The defect was reconstructed with an adjacent sternohyoid muscle flap in 3 control dogs. The surgical site was evaluated 3 months after operation by fiberscopic examination, computed tomographic imaging, histologic evaluation, and study of excised larynges. RESULTS: On fiberscopic examination, the experimental group implants were completely covered with regenerated mucosa in all cases, and a favorable vocal fold contour was found in 4 of the 5 cases. One case was characterized by a concave vocal fold shape and red granulation. In the control group, the muscle flap was replaced by scarred mucosa with a concave vocal fold contour in 2 cases, and there was soft white granulation at the anterior resected edge in the third case. The histologic data revealed the regeneration of lined epithelium, subepithelial tissue, and muscle structure in both groups. The excised larynx phonatory data revealed reduced vibratory amplitude in the experimental group compared with the control group; however, excised phonation was not achieved in 2 of the 3 cases in the control group. CONCLUSIONS: This polypropylene-based tissue engineering technique appears to be a viable tool for glottal reconstruction; however, additional refinement is required to maximize long-term phonatory function.

Full text

Objectives: The larynx must be resected in some cases of cancer or stenosis, and various techniques are generally employed to fill the resulting defect. No ideal way. however, has been established to restore vocal function after this form of insult. The aim of this preliminary feasibility study in a canine model was to investigate the effectiveness of a polypropylene-based tissue engineering approach to repair a partial glottal defect.

Methods: Eight dogs were used in this study. A laryngeal defect involving resection of the left vocal fold was created through a thyroid cartilage window. A scaffold made of polypropylene and collagen was preclotted and wrapped with autologous fascia lata, inserted through the window, and sutured to the laryngeal defect in 5 dogs. The defect was reconstructed with an adjacent sternohyoid muscle flap in 3 control dogs. The surgical site was evaluated 3 months after operalion by fiberscopie examination, computed tomographic imaging, histologic evaluation, and study of excised larynges.

Results: On fiberscopie examination, the experimental group implants were completely covered with regenerated mucosa in all cases, and a favorable vocal fold contour was found in 4 of the 5 cases. One case was characterized by a concave vocal fold shape and red granulation. In the control group, the muscle flap was replaced by scarred mucosa with a concave vocal fold contour in 2 cases, and there was soft white granulation at the anterior resected edge in the third case. The histologic data revealed the regeneration of lined epithelium, subepithelial tissue, and muscle structure in both groups. The excised larynx phonatory data revealed reduced vibratory amplitude