GTS

Abbreviations and Acronyms
CBF = cilial beat frequency
MRI = magnetic resonance imaging
MSC = mesenchymal stem cell

48 hours. The supernatant consisted of type 1 collagen (70%–80%), and the rest was type 3 collagen, as confirmed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. During the pepsin treatment, the telopeptide of collagen, which is thought to have antigenicity, was removed, leaving only the atelocollagen.

Prosthesis

A straight tube 16 mm in diameter was manufactured from a sheet of polypropylene mesh with a pore size of 260 × 10^{-6} m (Bard mesh; Davol Inc, Cranston, RI) by melting the edges together. The tube was then reinforced with a polypropylene monofilament yarn (0.8 mm in diameter) (Figure 1). The yarn was spirally attached to the external surface of the mesh tube by thermal melt-bonding at 5-mm intervals and further fixed with 7-0 Prolene polypropylene suture (Ethicon, Inc, Somerville, NJ). The polypropylene spiral provided the tube with stiffness against compression. The interval of the yarn was determined so that the mechanical resistance against compression was the same as that of the native trachea.[8]

This polypropylene tube was then exposed to a coronal discharge at 9 kV for 10 minutes to activate the surface. The prosthesis was subsequently coated 10 times with the 1% collagen solution. The activated prosthesis was covalently immobilized with collagen molecules and then further physically coated with collagen to promote host tissue incorporation and render the prosthesis airtight during the initial stage of implantation. This coating process was repeated 20 times.

The collagen-coated prosthesis was placed in a cylindrical Teflon mold with an inner diameter of 37 mm and a depth of 6 cm. At the same time, a 5-mm–diameter Teflon tube was inserted into the center of the prosthesis. The 1% collagen solution, which had been stirred at 8000 rpm for 15 minutes, was poured into the space between the outer mold and the inner tube and then freeze-dried. In this freeze-drying process, the cast collagen became a porous structure with a pore size range of 100 to 500 × 10^{-6} m. Finally, the prosthesis was subjected to dehydrothermal treatment 140°C in vacuo for 24 hours to induce cross-links between the collagen molecules to prevent early breakdown of the collagen in vivo.

Animal Experiments

Eighteen beagle dogs weighing between 8 and 14 kg were divided at random into three groups. The cervical trachea was cut transversely 20 mm caudal to the thyroid cartilage, and a tracheal segment (40–50 mm) including seven tracheal cartilages was removed with the animal under general anesthesia. During the operation, the prosthesis was preclotted with 5 mL of autologous peripheral blood in 8 of the dogs (peripheral blood group). In another 8 dogs, the prosthesis was soaked with 5 mL of bone marrow aspirate obtained from the femoral bone during the operation (bone marrow group). In the remaining 8 dogs, from which bone marrow aspirate had been taken and incubated in advance, the prosthesis was soaked with an incubated bone marrow cell suspension (MSC group). In these 8 dogs of the MSC group, 2 mL of bone marrow aspirate was taken from the femur 4 weeks before the operation. The bone marrow aspirate was incubated with Dulbecco modified Eagle medium containing 10% fetal bovine serum. The incubation medium was changed every 3 days from the fifth day. Floating cells were removed when the medium was changed. Some cells became attached to the bottom of the incubation bottle and proliferated. These proliferated cells were detached with trypsin, and a suspension of these cells (10^7) was used in the operating room.

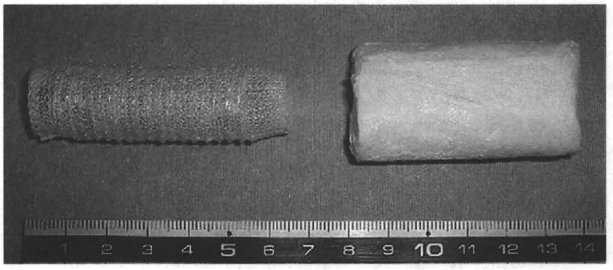

FIGURE 1. Mesh-type tracheal prosthesis. The framework is a polypropylene mesh tube reinforced with a spiral stent *(right)*. Freeze-dried collagen layers were made both inside and outside the framework as a scaffold of tissue regeneration *(left)*.

The porous collagen layer inside and outside the lumen shrank in volume on soaking. The prosthesis and the cut ends of the trachea were anastomosed with 3-0 Vicryl (Ethicon) interrupted sutures, the cut ends of the tracheal stumps being inserted into the prosthesis lumen (Figure 2). Neither omentopexy nor fibrin glue was used.

A 1000-mg dose of ampicillin was injected intramuscularly on the day of the operation, and a 500-mg dose was administered orally every day for 1 month thereafter. Bronchial cleaning with a fiberscope was only performed immediately after the operation on the same day. No additional care other than routine management was provided for the animals.

Bronchoscopic Examinations and Cilial Beat Frequency (CBF) Examination

A bronchofiberoscope (model BF1T20; Olympus Optical Co Ltd, Tokyo, Japan) examination was performed periodically under general anesthesia with an intramuscular injection of ketamine hydrochloride (10 mg/kg)

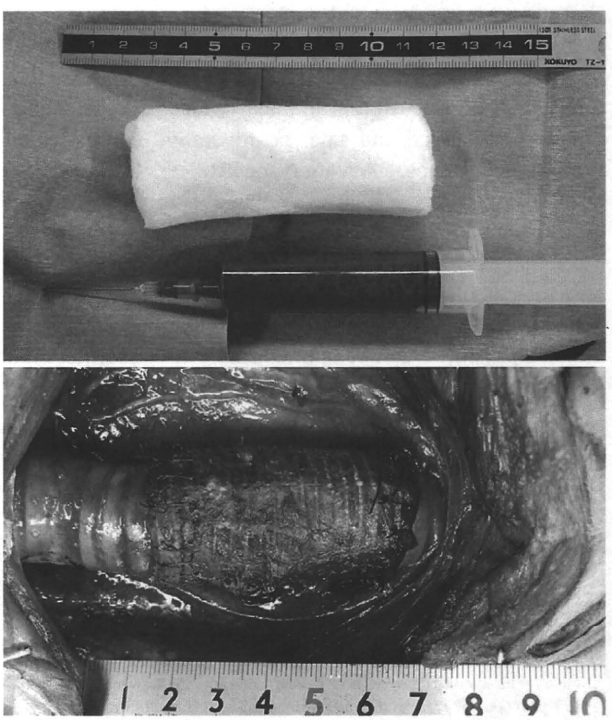

FIGURE 2. Intraoperative view of the reconstruction of a canine trachea with the prosthesis. The prosthesis was soaked with each medium before anastomosis. The prosthetic wall became airtight with this process.

TABLE 1. Results of tracheal reconstruction with prosthesis

Dog no.	Soak medium	Observation (mo)	Incorporation to host	Stenosis	Erosion
1	Peripheral blood	1	+	−	−
2	Peripheral blood	3	+	−	−
3	Peripheral blood	6	+	−	−
4	Peripheral blood	6	+	+	−
5	Peripheral blood	12	+	+	+
6	Peripheral blood	12	+	+	−
7	Bone marrow aspirate	1	+	−	−
8	Bone marrow aspirate	3	+	−	−
9	Bone marrow aspirate	6	+	−	−
10	Bone marrow aspirate	6	+	−	−
11	Bone marrow aspirate	12	+	−	−
12	Bone marrow aspirate	12	+	+	−
13	MSC suspension*	1	+	−	−
14	MSC suspension	3	+	−	−
15	MSC suspension	6	+	−	−
16	MSC suspension	6	+	−	−
17	MSC suspension	12	+	−	−
18	MSC suspension	12	+	+	−

Mesenchymal stem cells *(MSC)* were labeled with fluorescent marker DiI. *Stenosis is defined as a reduction of the tracheal lumen by more than one third.

and xylazine hydrochloride (4 mg/kg) to examine the luminal surface of the prosthesis. The replaced segments were examined carefully. Mesh exposure exceeding one third of the circumference was define as "++" and less than one third as "+." Stenosis was defined as a reduction of more than one third of inner luminal area.

At 6 months and 12 months, samples of the tracheal epithelium were removed with a brush inserted through the channel of the bronchoscope to evaluate cilial movement. The brush, which scrubbed the target (reconstructed) portion of the tracheal mucosa, was washed with culture medium. This medium (containing the scrubbed tracheal mucosa cells) dropped on a glass slide was then observed with a light microscope. The movement of the cilia on the slide was counted at 37°C with a photomultiplier system[9] connected to the microscope.

Cilial movement frequency was compared among the three groups by analysis of variance and a *t* test. All frequency measurements obtained are expressed as the mean (± standard deviation) of at least 10 determinations.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) of the reconstructed trachea and adjacent organs was conducted on 1 dog from each group 12 months after implantation. The MRI system used was a Sonata MRI System with a static magnetic flux density of 1.5 T (Siemens Medical System, Erlangen, Germany) using a phase-array body coil with four active segments. The dog was anesthetized with an intravenous injection of propofol (Diprivan; 5 mg · min^{-1} · kg^{-1}) during the imaging.

Histologic Examination

One dog in each group was humanely killed with an injected overdose of sodium pentobarbital 1 and 3 months after tracheal reconstruction. Two dogs in each group were humanely killed 6 months after tracheal reconstruction. At 12 months, 2 dogs in the peripheral blood group and 1 each in the bone marrow and MSC groups were humanely killed. The remaining dog in each of the bone marrow and MSC groups was allowed to survive for further observation.

En bloc resection of the prosthesis with the native trachea was carried out for histopathologic evaluation. The epithelial cells on the inner surface were checked with a scanning electron microscope (S-450; Hitachi Ltd, Tokyo, Japan).

All of the surgical and euthanasia procedures were performed in accordance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH Publication No. 85-23, revised 1985). The experimental protocol was approved by Animal Experimental Committee of Kyoto University.

RESULTS

All animals in the three groups had uneventful postoperative courses, and no symptoms that necessitated unscheduled humane killing of an animal occurred (Table 1).

Bronchoscopic Examination

Bronchoscopic examinations at 1 month revealed that the luminal surface of the implanted prosthesis appeared glossy and whitish, suggesting complete coverage of the lumen by regenerated tissue (Figure 3). No mesh exposure was recognized even in the middle of the prosthesis, except in 1 dog (Table 1, dog 5), in which an area of exposed mesh measuring about 5 × 2 mm was observed at the center of the prosthesis at 1 month. Despite this mesh exposure, the dog was asymptomatic and its general condition was good, and no retention of sputum was observed in the lumen of the prosthesis up to 12 months. Stenosis of the prosthetic lumen was observed in 3 of the dogs in the peripheral blood group and in 1 dog in each of the other two groups. This stenosis occurred in the middle of the prosthesis and not at the sites of anastomosis. The definition of stenosis used in this study was reduction of the tracheal luminal diameter by more than one third. Whereas both dogs in the bone marrow group and the MSC group had only mild stenosis, severe stenosis that reduced the diameter of the tracheal lumen by more than half occurred in 3 of the 4 dogs in the peripheral blood group. The narrowest luminal diameter was 9 mm in both the bone marrow group and the MSC group, whereas that in the peripheral blood group was 6 mm.

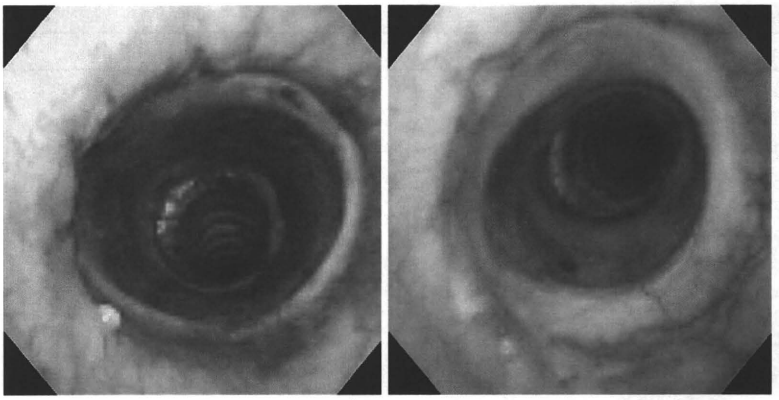

FIGURE 3. Bronchoscopic views of the reconstructed site of the bone marrow group (dog 11) 1 month *(left)* and 12 months *(right)* after the operation. Neither stenosis nor granulation is evident at the site of anastomosis with the trachea. Complete coverage of the mesh by regenerated tissue is evident.

Macroscopic Examination

At 1 month the inner surface of the prosthesis was covered with glossy tissue in all three groups (Figure 4). The prostheses were incorporated into the native tracheas, and there were no major complications such as erosion of the surrounding organs, abscess formation, pneumothorax, anastomotic dehiscence, or prosthetic dislocation in any of the 18 dogs that were humanely killed on schedule (Table 1).

MRI

MRI at 12 months indicated no scar formation around the prosthesis, and the inner lumen of the tube was maintained in the cervical space in all three groups (Figure 5). The implanted prostheses were well incorporated with the native trachea at the interfaces, and there was no morbid tissue reaction between adjacent organs including major blood vessels.

Histologic Examinations

Examination with a light microscope at 1 month revealed that the polypropylene mesh and polypropylene spiral had become buried in newly regenerated connective tissue and that the implanted collagen had disappeared. Capillary ingrowth was observed in the regenerated tracheal tissue in all dogs of the three groups. After 6 months, formation of respiratory epithelium was confirmed histologically from the upper to the lower anastomotic site of the prosthesis (Figure 6).

Although the extent of the epithelial lining of the regenerated mucosa on the prosthesis varied, 9 of the 12 dogs in the bone marrow and MSC groups had an epithelial lining covering more than 50% of the length of the prosthesis. In contrast, after 3 and 6 months, the peripheral blood group had poor epithelial lining, in which the squamous epithelium was thicker than that of the columnar bronchial epithelium. After 12 months, the extent of the epithelial lining in the peripheral blood group was similar to that in the other two groups. In each animal, ciliated columnar epithelium was observed near the anastomoses, the proportion of nonciliated cuboidal epithelium increased with distance from the anastomoses, and squamous epithelium was observed in the center of the prosthesis (Figure 7).

An area of exposed mesh measuring 5 × 2 mm without any sign of local infection was observed in dog 5 of the peripheral blood group. No granulation tissue formation was detected in the lumen.

Histologic examination of the stenotic part of the prosthesis in 5 dogs showed that the connective tissue, including vessels, had invaded the mesh pores, covering the inner surface of the prosthesis. Also in these animals, the neoepithelium appeared stretched from the cut end of the tracheal stump over the connective tissue layer.

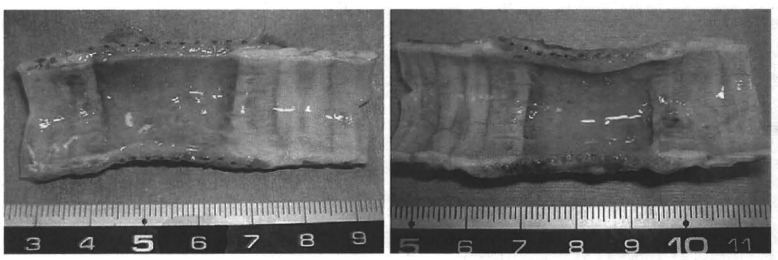

FIGURE 4. Luminal surface of the prosthesis (bone marrow group, dog 8). Three months after reconstruction, the prosthesis was incorporated into the native tracheas and inner surface was covered with glossy and whitish tissue. Polypropylene stents were seen in the cross-section of the reconstructed tracheal wall.

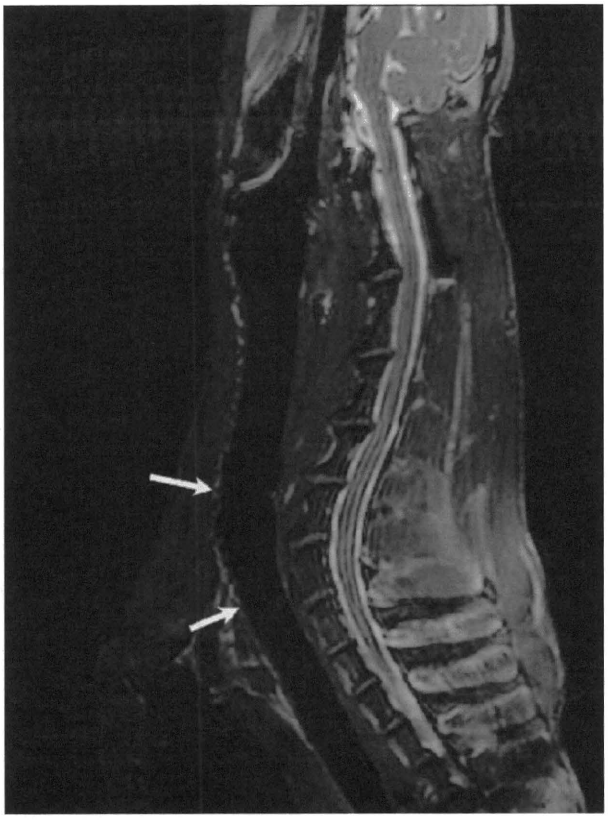

FIGURE 5. MRI appearance of the prosthesis implanted into the cervical trachea of a dog 12 months after the operation (MSC group). Replaced area is indicated by the *arrows*.

Scanning electron microscopy examination revealed that the regenerated epithelial cells near the anastomoses possessed cilia similar to those of the normal tracheal epithelium in all groups. Moreover, a few ciliated epithelial cells were observed in areas where only a squamous cell lining was demonstrated by light microscopy (Figure 7).

Observation by transmission electron microscopy showed long and uniform cilia on the luminal surface over a large area, gathering closely together (Figure 8) on the regenerated part of the trachea in all three groups. In the area where ciliated cells were absent, the luminal surface was covered with the squamous cells.

Comparison of CBF in the Regenerated Mucosa

CBF in the regenerated tracheal mucosa is shown in Figure 9. CBF of the same portion of the trachea measured in intact normal beagle dogs using the same system was 14.6 ± 3.5.

At 6 months, CBF values in the bone marrow group and MSC group had recovered to almost the normal range and were significantly higher than that in the control group. There were no significant differences in CBF among the three groups at 12 months ($P > .05$).

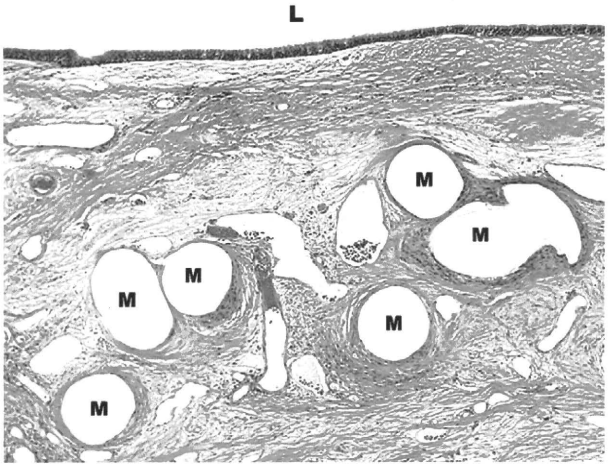

FIGURE 6. Regenerated mucosa with epithelial lining on the mesh of the prosthesis from a dog in the bone marrow group after 12 months. The mucosa is thicker than the normal tracheal mucosa. *L*, Tracheal lumen; *M*, mesh of the prosthesis (hematoxylin and eosin staining; original magnification, ×400).

Proliferation of Bone Marrow–Derived Cells

To determine the fate of the MSCs, we used MSC labeled with Di-I (Cell Tracer CM-DiI; Molecular Probes, Leiden, The Netherlands) in 1 dog (no. 13) of the MSC group, which was killed 1 month after the operation. At this time, the Di-I–labeled MSCs were recognized in the regenerated tracheal tissue. They remained in the submucosal tissue around the polypropylene mesh and were not present in the mucosa (Figure 10).

DISCUSSION

Direct end-to-end anastomosis has been the "gold standard" for airway reconstruction.[10] However, the range of possible reconstruction with this method is limited, and even within the feasible range (less than 6 cm), tension-reducing procedures are required during the operation. In the case of resections approaching the theoretical maximum, patients must endure a stressful drawn-in chin position with neck fixation for at least 2 weeks after the operation to avoid concentrating tension on the sites of anastomosis.[2] Thus, direct apposition and suturing of the tracheal stumps tends to place a heavy burden on the patient. For resolution of such problems, the use of a tracheal substitute makes reconstruction easier, and as a result, the surgical approach can be expanded with the use of a tracheal prosthesis.

Many types of material have been examined for use in tracheal prostheses since the first report by Daniel[11] in 1948. A prominent milestone in the history of artificial trachea development was the introduction of a silicone rubber prosthesis in the 1960s, which became commercially available in the 1970s[12] and was used clinically for a short time. However, because re-epithelialization could not be expected on the

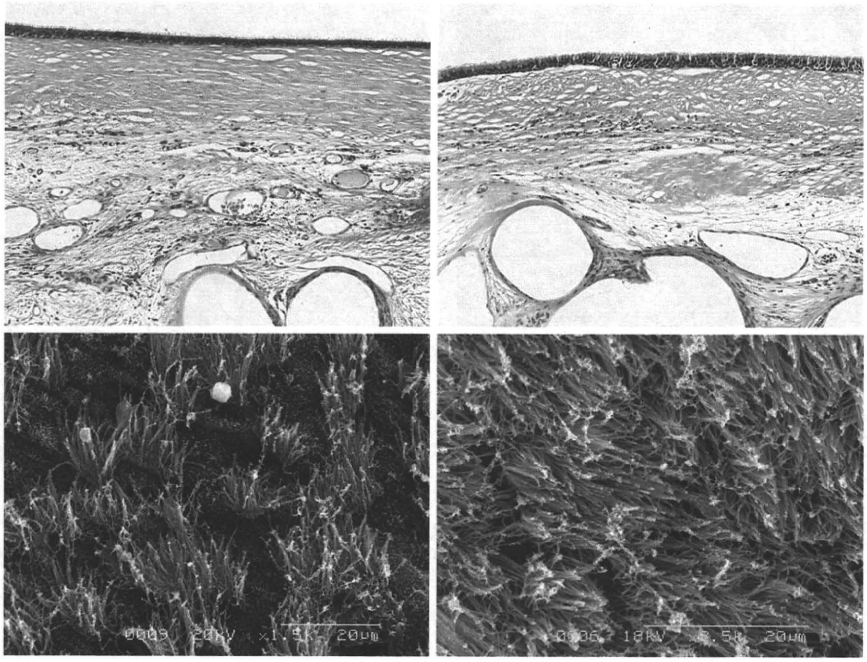

FIGURE 7. Ciliated epithelium is present on the regenerated mucosa near the anastomosis *(upper left)*, but only squamous epithelium is present in the middle of the prosthesis *(upper right)* in a dog in the bone marrow group after 12 months (hematoxylin and eosin staining; original magnification, ×200). *Bottom,* Scanning electron microscopy (SEM) views of the luminal surface. Dense cilia like those of the normal tracheal epithelium are present on the prosthesis near the anastomosis *(lower left)* and a few ciliated epithelial cells are present in areas where only a squamous cell lining was demonstrated by light microscopy *(lower right)*. Although these findings were evident in all three groups, there were more ciliated cells in the bone marrow and MSC groups than in the peripheral blood group.

inner lumen, formation of granulation tissue or dehiscence occurred at the interface between the prosthesis and the native trachea, usually within several months.[13] For this reason, the nonporous tracheal prosthesis is now seldom used clinically.

One of the reasons that prosthetic tracheal reconstruction is more difficult than use of a vascular prosthesis may be that the trachea is in contact with the external environment (ie, it is an airway). At the interface of an implanted foreign body and host tissue, the wound healing process continues until the implanted foreign body is completely encapsulated or is rejected from the implant site. Such phenomena are often observed clinically by surgeons in relation to suture materials used on skin incisions. In this respect, an artificial trachea contrasts conspicuously with an artificial vascular graft, which has no external interface after implantation.

With this in mind, we[3,4,7,8,14] have designed a series of mesh-type tracheal prostheses that eliminate the interface facing the airway. In such prostheses, autologous tissue invades the pore spaces, and the interface of the foreign material with the airway finally disappears with re-epithelialization of the inner lumen.[15]

However, one problem with the mesh-type prosthesis that needs to be overcome is insufficient air sealing of the mesh structure, especially just after implantation. Indeed, to seal the porous tracheal prosthesis, a 2-stage operation was once designed for ensuring that the prosthesis was airtight.[16,17] However such a 2-stage operation places a heavy burden on the patient and seems too complicated for clinical use.

Therefore, to seal the mesh, we[14] began to apply collagen in the early 1980s in the expectation that the collagen might act not only as a palliative sealant but also as a scaffold for tissue regeneration. Collagen, the main element of the extracellular matrix, has been reported to play several important roles in tissues, such as cell adhesion, detachment, transformation, and proliferation.[18] For example, type 1 collagen is reported to promote the differentiation of tracheal ciliated epithelium.[19]

In this connection, tissue engineering, whereby a variety of tissues can be fabricated in incubation rooms using cell culture techniques, has been a focus of attention since 1990.[20] Tissue engineering has also been applied for tracheal reconstruction. In 1994, epithelial tissue was first induced on a tissue-engineered trachea.[21] In that study, tracheal epithelial cells were isolated from newborn lambs in advance to induce an epithelial lining on the reconstructed site. These epithelial cells were injected into a previously manufactured cylindrical cartilage tube made from cells harvested from calves. These in vitro–formed tubes were then implanted into subcutaneous pockets of 10 nude mice, aged 6 weeks. Four of the 10 implanted tubes were found to develop an epithelial lining on the inner surface, whereas

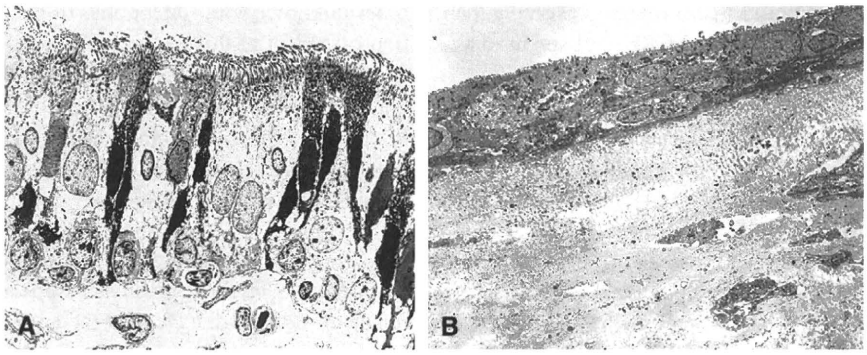

FIGURE 8. Transmission electron microscopic view of the regenerated mucosa of the reconstructed segment (center) 12 months after reconstruction in the MSC group (A). In the area where ciliated cells were absent, the luminal surface was covered with the squamous cells (B).

the other 6 tubes became infected. In 2001, in vitro–cultured tracheal epithelial cells were successfully transplanted onto a prefabricated capsule pouch surface with fibrin glue for possible use in tracheal reconstruction.[22]

Development of tracheal cartilage has also been studied in rats by means of tissue engineering.[23] Kojima and coworkers[24] have successfully constructed an autologous tissue–engineered trachea with sheep nasal chondrocytes and used it to reconstruct the trachea of 6 sheep. However, because of malacia and stenosis, their animals survived only 2 to 7 days after surgery. Okamoto and coworkers[25] have made tracheal cartilage using bone morphogenetic protein 2 in dogs. In their canine model, a 1 × 5-cm slit defect of the tracheal cartilage was created in the trachea in advance, while carefully preserving the tracheal mucosa. Subsequently, regeneration of the cartilage was recognized around the stumps of the resected cartilages. Although these tissue engineering trials have met with some limited success, achievement of a long-term durable tracheal cartilage with mechanical properties similar to those of native tracheal cartilage still seems a long way off.

In situ tissue engineering is a novel method wherein tissue is formed not in vitro previously, but in the patient's body.[26] That is to say, in conventional tissue engineering, a required tissue has to be made previously on an incubation dish in the laboratory room. By contrast, in in situ tissue engineering, a scaffold of tissue is placed directly at the site of a tissue defect in the body where regeneration of the tissue is required.

In our early experiment, in which a prototype Y-shaped tracheal prosthesis was simply coated with amorphous collagen alone, 13 of 20 dogs died within 11 days after tracheal reconstruction.[7] The main reason for these early postoperative deaths was air leakage from the trunk of the prosthesis. To address this problem, we[27] improved the structure of the tracheal wall by increasing the amount of collagen through introduction of a porous collagen layer that was laid over the amorphous collagen layer. This improvement made the surgical procedure easier, and neither fibrin glue nor pledget sealing was necessary at the anastomosis sites during the operation.

For evaluation of functional recovery of the reconstructed tracheal wall, we measured the CBF of the regenerated

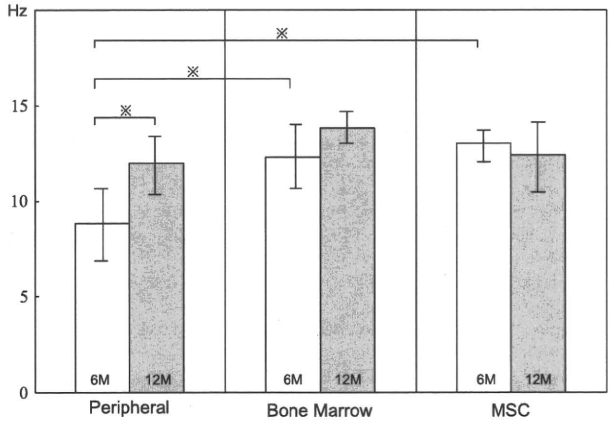

FIGURE 9. Cilial beat frequency of the regenerated cilia. *MSC,* Mesenchymal blood cell.

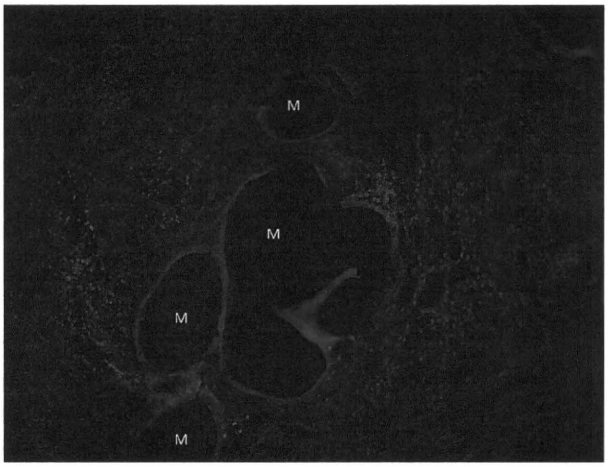

FIGURE 10. Microscopic appearance of the regenerated part of the tracheal wall after 1 month, showing numerous seeding MSCs, which are labeled with FM-DiI fluorescent tracer (original magnification, ×100). The labeled cells remained in the submucosa tissue around the polypropylene mesh and did not exist in the mucosa. *M,* Mesh of the prosthesis).

tracheal epithelium. Cilia play a major role in preserving the functional integrity of the airway, and CBF has been used as a marker of tracheal function in the field of pulmonology.[28] The CBF value of the regenerated cilial area was 80% to 90% of the normal value in the bone marrow and MSC groups at 6 months and significantly higher than that in the peripheral blood group, although by 12 months the difference among the three groups was not significant. This may indicate that the reconstructed area had recovered not only morphologically but also functionally.

In our previous series of experiments, we[2-6] had soaked the collagen sponge of the prosthesis with peripheral blood during the operation. Because collagen has high affinity for platelets,[29] we expected that the platelets attached to the collagen might provide platelet-derived growth factor or other active molecules that would help to enhance tissue regeneration. In the present study, in addition to peripheral blood used previously, we applied two other types of soaking medium: a bone marrow aspirate and a suspension of incubated bone marrow–derived cells. The latter cells have a tendency to attach to the bottom of the incubation bottle. Inasmuch as these cells have the potential to differentiate into several types of mesenchymal tissue, such as muscle, fat, and bone, they have been called multipotential bone marrow–derived cells or MSCs.[30] These cells have already been applied clinically as a cell source for tissue engineering.[31]

The results obtained in the bone marrow aspirate and MSC groups were better than those in the peripheral blood group. Mesh exposure and luminal stenosis occurred in only 2 dogs of the former two groups. These complications were milder in degree than those in the peripheral blood group. There was no significant difference in performance between the bone marrow aspirate group and the MSC group. In contrast, in the peripheral blood group, small areas of either mesh exposure or luminal stenosis occurred in all of the dogs but did not threaten the general condition of the animals or cause any significant problems. Evaluation of the functional recovery of the reconstructed site in terms of cilial movement revealed a similar tendency. Therefore, the application of bone marrow and MSCs to our prosthesis seemed to facilitate healing of the reconstructed tracheal region.

In the present study, we found that the implanted MSCs proliferated into the tracheal tissue. However it still remains unclear whether the cells in the soaked bone marrow aspirate also proliferated into the tracheal tissue. It will also be necessary to carry out further studies of the mechanism whereby soaked cells assist in tracheal remodeling on the collagen scaffold.

From a clinical viewpoint, use of a patient's own bone marrow aspirate seems to have many potential advantages. Aspiration of 2 mL of bone marrow from an anesthetized patient is relatively easy in the operating room and requires no incubation process to encourage cell proliferation. Eliminating the use of fetal bovine serum also obviates the risk of other unknown viral infections. In this regard, the application of MSCs in this experiment or iPS (induced pleuripotent) stem cells drawing current interest of the world have the same disadvantage; their clinical safety in terms of virus infection or tumorigenicity has not been established, inasmuch as fetal bovine serum or other nonautologous substances are now indispensable for preparation of such MSCs as well as iPS (induced pleuripotent) stem cells.[32]

In conclusion, although further long-term observation is required, our tracheal prosthesis appears promising for the repair of tracheal defects, and the application of autologous bone marrow may be clinically most feasible to assist the regeneration of tracheal tissue.

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The Laryngoscope

Chronic Vocal Fold Scar Restoration With Hepatocyte Growth Factor Hydrogel

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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.

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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]

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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).

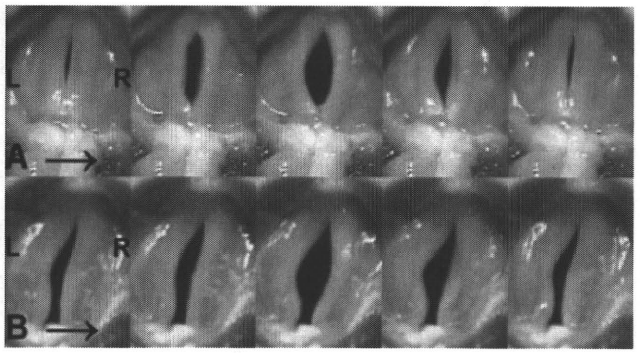

Fig. 1. Vibratory patterns experimentally generated from treated vocal folds of excised larynges. In both cases the left vocal fold was scarred. The hepatocyte growth factor-treated vocal fold showed almost normal mucosal vibration (A), however the sham-treated vocal fold was bowed and mucosal vibration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

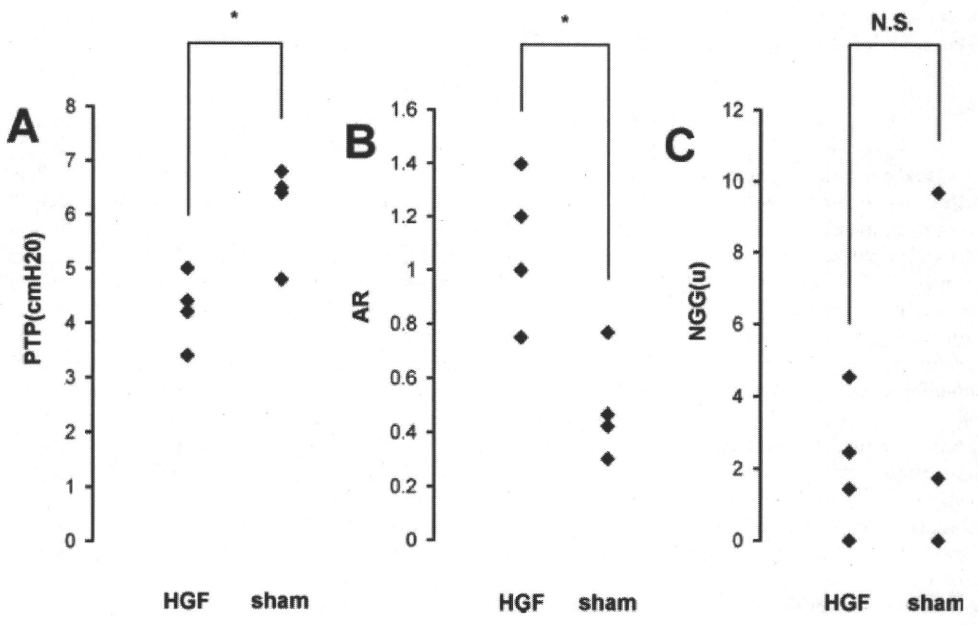

Fig. 2. Results of vibratory examinations. The hepatocyte growth factor (HGF)-treated group demonstrated significantly lower phonation threshold pressure (PTP) (A) and higher amplitude ratio (AR) (B) compared with the sham-treated group. No differences were observed for normalized glottal gap (NGG) between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

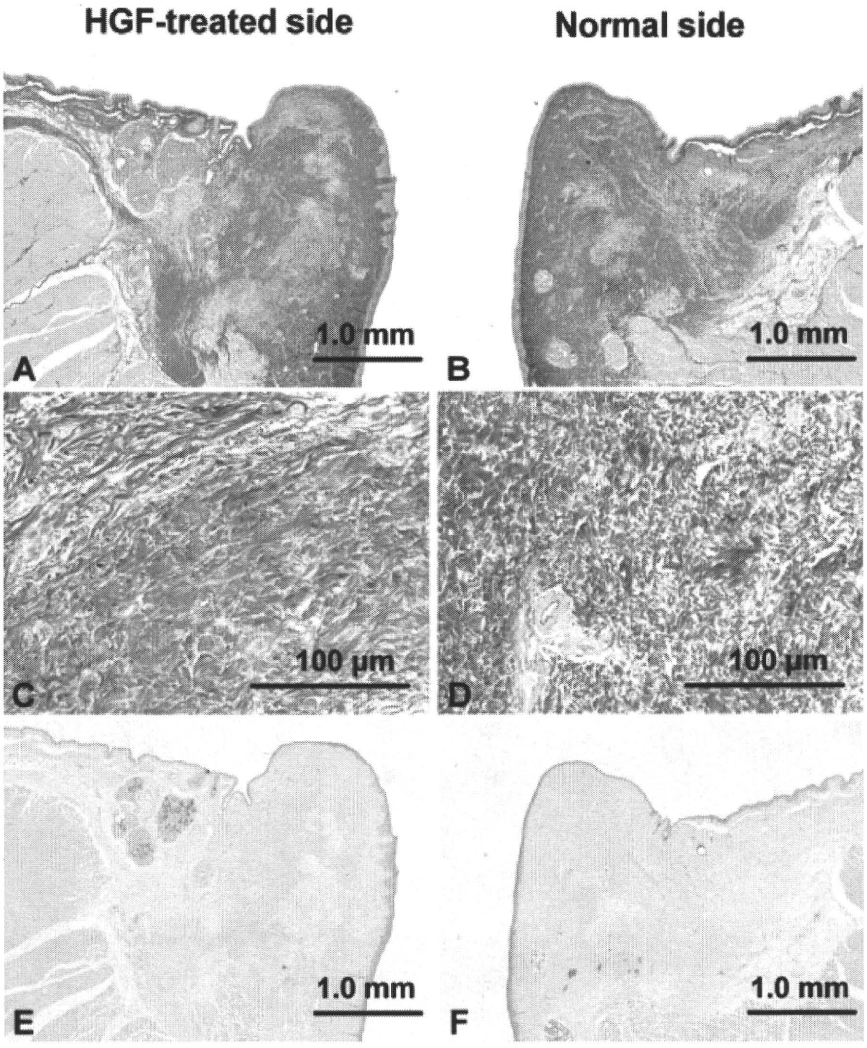

Fig. 3. Histologic findings in the hepatocyte growth factor (HGF)-treated group. (A–D) Elastica van Gieson stain. (E–F) Alcian blue stain. Tissue contraction and collagen deposition were found to be minimal (A, B), and elastin (C, D) and hyaluronic acid (E, F) were favorably restored.

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

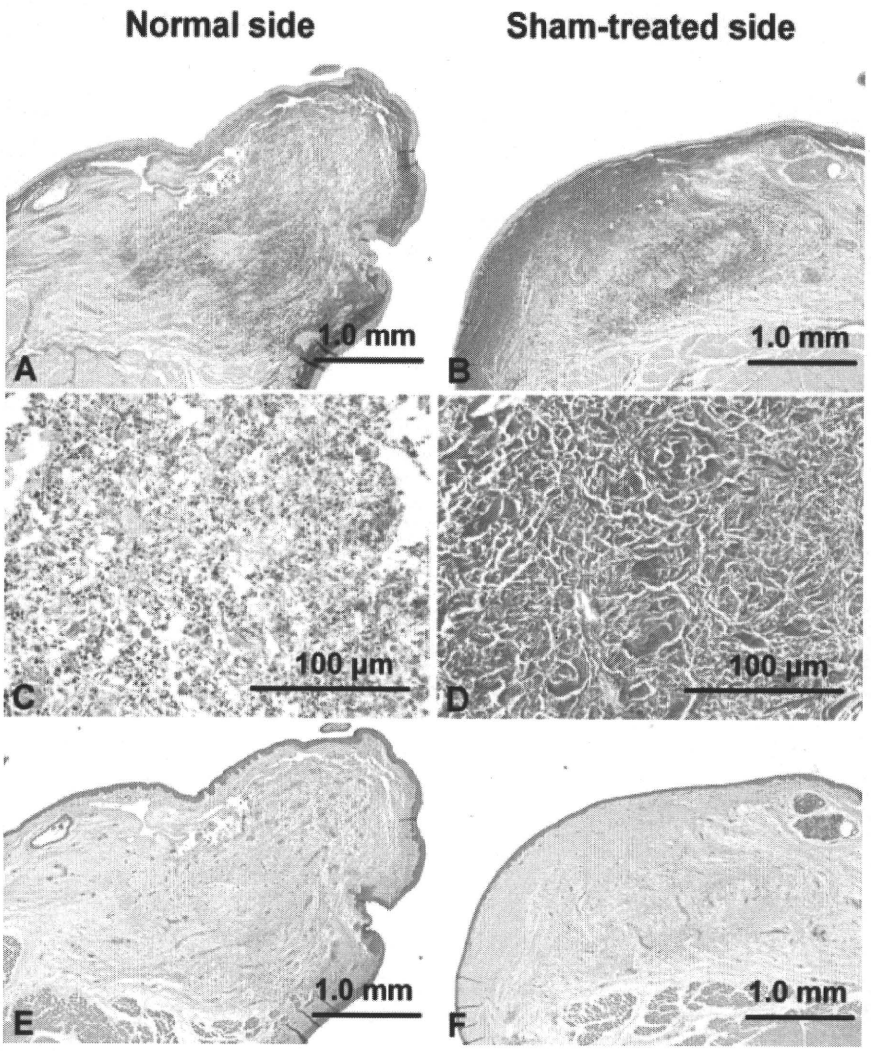

Fig. 4. Histologic findings in the sham-treated group. (A–D) Elastica van Gieson stain. (E–F) Alcian blue stain. Severe tissue contraction and excessive collagen deposition were observed in sham-treated vocal folds (A, B). Elastin (C, D) and hyaluronic acid (E, F) were decreased in the superior portion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

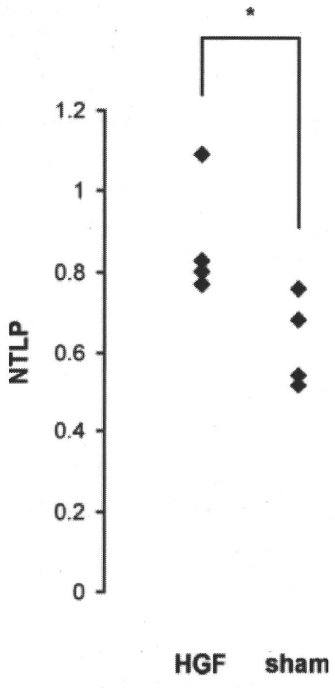

Fig. 5. Normalized thickness of lamina propria (NTLP) was significantly thinner in the sham-treated group than in the hepatocyte growth factor (HGF)-treated group. *$P < .05$.

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.

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21回日本末梢神経学会学術集会　シンポジウム2「末梢神経の再生」3

人工神経の基礎と臨床*

中村　達雄[1)], 萩原　明於[1),2)], 稲田　有史[1),3)], 金丸　眞一[4)]

Key Words: artificial nerve（人工神経）, nerve conduit（神経チューブ）, regeneration（再生）, collagen（コラーゲン）, in situ Tissue Engineering（生体内再生）

Peripheral Nerve 2010; 21(2): 192–196

はじめに

19世紀に神経解剖学の基礎を確立したカハール（Santiago Ramón Y Cajal）がいみじくも指摘したように、中枢神経はほとんど再生しない。これとは対照的に末梢神経は旺盛な再生能がある。両者の再生能の違いはどこから来るのだろう。全く著者の想像ではあるが、中枢神経は記憶をはじめとする高度な機能をもった臓器であり、頑丈な頭蓋骨や脊椎で守られている。つまり傷つかないように厳重に守られているかわりに再生しないのではないか。というのも、脳が記憶機能に関与しているために、損傷を受けた後むやみに再生するとこの記憶が混乱してしまうからではないか。胸郭に守られている心臓や肺に再生能がなく、骨性胸郭に守られていない肝臓に旺盛な再生能があるのもこれに近い生体の設計戦略によるのかもしれない。中枢神経系では、損傷が起きるとグリア細胞がNOGOやNI-35をはじめとした種々の軸索再生阻害因子を分泌するシステムが存在する。つまり再生しない仕組みがあらかじめ中枢神経には組み込まれているのである。

これに対して、末梢神経は四肢の末梢と中枢を結ぶ情報の導線として全身に張り巡らされるという解剖学的な特徴がある。したがって構造上、全てを骨組織で守ることは不可能である。そこで切れた場合にも、回復するメカニズムが付与されているのではなかろうか。この末梢神経の自己再生（修復）能力を利用した治療法の1つとして、人工神経（神経連結管）が開発されている。ここでは、人工神経の基礎と臨床について紹介する。

1．末梢神経の再建法

事故や外傷で末梢神経が切れた場合、切断端間の距離が5mm以内であれば直接縫合が可能である。しかし、ギャップが5mm以上ある場合、末梢神経組織は長軸方向の伸展に弱いので吻合部に強い張力がかかるため、断端を引き寄せて吻合しても機能が戻らない。そういった5mm以上の欠損に対して、従来は自家

* Artificial nerve (Nerve conduit): Research and clinical application

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神経移植がGOLD STANDARDの術式として行われてきた。

自家神経移植で使う神経は末梢神経の中でも切除しても比較的術後障害が少ない感覚神経が選ばれ、これを採取して、再建部に移植するものである。よく用いられるのは下肢の腓腹神経、耳鼻科領域では大耳介神経である。この自家神経移植という術式は「移植」という言葉が使われるが、肺臓移植や心臓移植など他の臓器移植とは概念が大きく異なる。すなわち自家神経移植では移植された自家神経片は神経軸索として機能するのではなく、移植片は神経再生の足場として働く。主な機能をつかさどる神経軸索は切断中枢端から伸展し、移植された神経片を貫通してWaller変性した末梢端に入り、さらにそこから最終標的器官である神経筋接合部や感覚受容器に到達して初めて神経機能が回復する。

マイクロサージェリー技術の発達により、微細な神経縫合が可能になり、自家神経移植は標準術式になった。しかしながら自家神経移植には必然的に犠牲にする神経がどうしても必要であり、また術後の機能回復成績も決して満足するものではなかった。さらには神経片採取部位に新たに疼痛を生じた症例も報告されている。

2．人工神経の歴史

人工神経が開発される以前は、自己の静脈管を用いて神経欠損部を補填する試みが1904年頃から行われた[1)]。1970年代から自家神経移植に代わる新しい術式として、チューブによる欠損部の補填が研究されてきた。切断された神経の両端をチューブで連結しておくと、両端から組織が伸びてきて、チューブ内に連続した索状組織ができる。そしてその新生組織内を中枢端から軸索が伸展する。この現象に注目して神経連結管（人工神経）の研究を大きく発展させたのは本学会でも平成18年8月に広島で特別講演をされたスウェーデンのGöran Lundborg博士と同僚のDahlin博士のグループである。Lundborg博士らはシリコン製のチューブを用いて神経を再生させることに成功し、さらに臨床に応用した[2)]。しかしながら、シリコンに代表される非吸収性材料のチューブは再生した神経にとっては周囲との隔壁であり、再生部位の疎血の原因となる。従って長い距離の再建に用いると機能的回復が遅れる。そこで神経がつながった後、チューブは手術で二期的に抜去する必要があるが、これは患者にとっても外科医にとっても大きな負担であった。

3．生体内吸収性人工神経

抜去手術の不要な神経連結管（人工神経）として分解吸収性チューブの開発が進められた。生体で安全に分解吸収する外科用埋植材料としてはポリグリコール酸（PGA）、ポリ乳酸やこれらの共重合体が1950年代から吸収性外科用縫合糸として使われている。これらの素材で作られた神経管が開発された。アメリカでは1990年代終わりにすでにNEUROTUBE（図1）という商品名のPGA製人工神経が開発され、FDAの承認を得て、30mm以内の欠損補填では自家神経移植に比べて良好な感覚機能の回復が得られることが、ランダムスタディで証明されている[3)]。また、

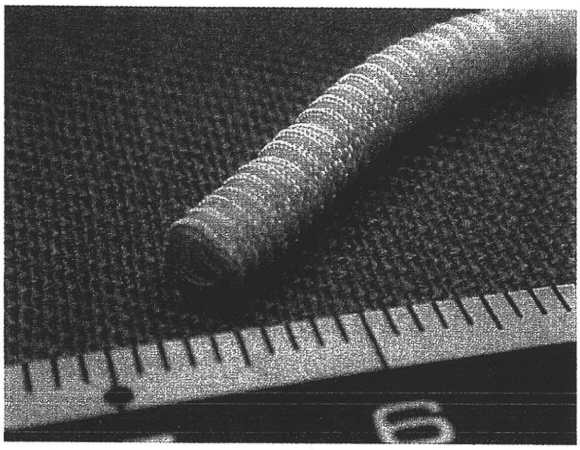

図1　アメリカで販売されているPGA製人工神経（Neurotube）

表1　Nerve Guide Tube commercially available

	Product Name	Company	Country	Material	FDA510k	φ (mm)	L (mm)
1	Neurotube	Synovis LT	USA, MN	PGA woven corrugated tube	1999	2.3～8	20～40
2	Neurolac	Polyganics BV	Netherlands	poly (DL-lactide-co-e- caprolactone)	2003	1.5～10	30
3	NeuroGen*	Integra NS	USA, NJ	Collagen	2001	2～7	20～30
4	Neuroflex	Stryker/Collagen Matrix	USA, NJ	Type I collagen	2001	2～6	25
5	Salu Bridge	Salumedica	USA, GA	Poly (vinyl alcohol) hydrogel	2000	2～10	64
6	Axo Gen	Cook Bioteck	USA, IN	porcine small intestine	2003	1.5～7	10

*Integra provides now NeuraGen tube, similar to previous NeuroGen.

生体内由来物質であるコラーゲンを架橋した素材で作られた人工神経管も、NeuraGenという商品名で市販されている。この他に同様の中空の連結管が著者らの知る限り全部で7種類ほどFDAの認可を得ている（表1）。

4．人工神経の内部構造

現在製品化されている人工神経はアメリカ製とオランダ製のものがあるが、日本では市販されていない。いずれも中空な構造である。使用に際しては内部に生理食塩水を充填して使うように使用説明書に記載されている。

神経管内部に神経の伸展を促進する物質を補填する研究が進められている。

神経組織は基底膜のIV型コラーゲンに沿って伸展する性格がある。そこでIV型コラーゲンやラミニンを充填したり、また神経成長因子（NGF）を応用したりする方法が考案されている[4]。さらに培養したシュワン細胞を神経管内に充填して軸索再生を促進させる試みも進められている[5]。さらに未分化な体性幹細胞をシュワン細胞に分化させてこれを用いる手法も研究が進められている。

神経管の内部では軸索が長軸方向に向かって伸びる必要があり、伸びるための足場となる線維束を長軸方向に充填する実験が行われた。この足場にはコラーゲン繊維や生体内分解性合成高分子材料の繊維などが使われる。

コラーゲンの繊維束を入れた人工神経は長い欠損においても良好な神経再生をもたらすことが判明した。しかしこのコラーゲン繊維は一定の品質の繊維を作るのが難しく、作製には大がかりな紡糸装置が必要で作成費が極めて高価になる。この点が人工神経を製品化するには大きな障害になっていた。ところが、充填物は繊維束でなくても凍結乾燥させたコラーゲンを充填しても同等の神経再生効果があることが判明して[6]、人工神経の開発は大きく進歩した。人工神経管内で凍結乾燥させたコラーゲン水溶液は薄フィルム多房構造（図2）となり、これが神経の再生に良好な環境を作り出すからである。

5．新しく開発された人工神経（PGA-Collagen Tube）とその臨床応用

米国製の人工神経管が中空であったのに対して、日本で開発された人工神経は内部にコラーゲンが充填されている点に特色がある。軸索の再生の場としての足場の利用である。

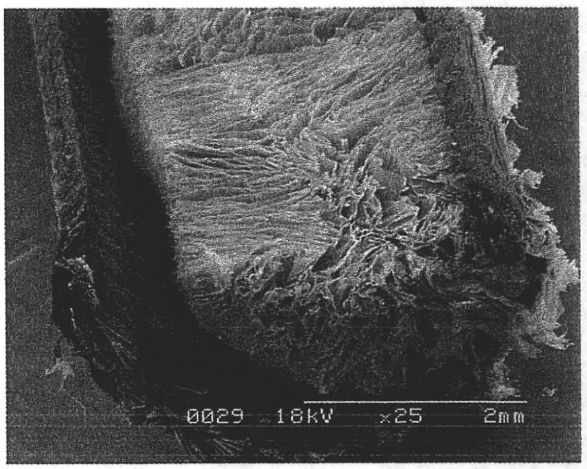

図2　PGA-Collagen Tube内コラーゲンの薄フィルム多房状構造（SEM所見）

組織工学では足場、細胞、増殖因子を駆使して組織を作製するが、このタイプの人工神経では再生の場を培養室のシャーレの中ではなく直接再生部位に置くことにより、局所にある生体由来の増殖因子、細胞などの力を使って神経を再生させるもので、生体内組織再生（in situ Tissue Engineering）の応用である。

ビーグル成犬を用いた動物実験で80mmの欠損で神経再生が確認され[7]、また自家移植との比較でも腓骨神経15mm欠損を補填すると、電気生理学的にも組織学的にも自家神経移植に比べて良好な回復をすることが判明した[8]。

そこで、このPGA-Collagen Tube（図３）は2002年より京都府立医科大学で学内の倫理委員会の承認のもと、初めて臨床応用が行われた[9]。さらに現在、奈良県立医科大学、稲田病院、京都大学病院、田附興風会北野病院、新潟大学歯学部でも臨床応用が続けられている。

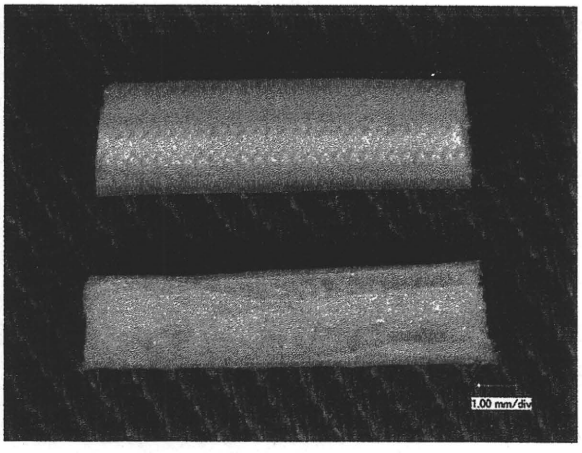

図３　PGA-Collagen Tube

６．人工神経の適応疾患と再生の場の理論

人工神経の臨床応用では、外傷や医原性の神経断裂が主なる治療対象になる。これ以外には悪性腫瘍切除後の神経再建にも使われている。人工神経は末梢神経の自己再生能力に依存しており、極端に血流が乏しい部位や、放射線照射が予定される部位では使用できない。生体内組織再生（in situ Tissue Engineering）では、その場に再生に適した環境（再生の場）を作り出すことが必要だからである。

受傷から神経再建までの時間は早いほど良いことは言うまでもない。しかし複雑骨折などを伴った外傷例などでは周囲の創が治癒するのを待って神経再建手術が行われることも多い。受傷後数年以上経った陳旧症例の再建にも用いられているが、やはり新鮮例に比べると回復は遅く、受傷後１～２年以内の症例が良い適応と考えられる。

７．人工神経の埋入手技

神経の両端が神経管内に内挿されるように8-0モノフィラメント糸で固定する。この際に神経の断端がチューブ内で向き合うように固定することが大切である。

何度も繰り返すが、周囲に血流豊富な軟組織があり、瘢痕を作らないような環境が望ましい。このために血管柄付組織移植などのマイクロサージェリーの技法を併用して、周囲の再生環境を整えることが必要になる症例もある。

８．人工神経（PGA-Collagen Tube）による臨床の実際

人工血管と人工神経とは名前は似ているが、臨床像が全くと言ってよいほど異なる。血管再建手術では、縫合が完了してクランプを外した瞬時に血流が再開し機能は回復する。人工神経では縫合が完了しても機能的には全く改善が見られない。軸索が伸長して目的組織に到着して初めて機能が回復する。そのため機能回復に数年かかることも珍しくない。また術後の神経再生過程で異常感覚や局所の再生痛が生ずることもある。

この点を術前に患者に説明し、理解の得られた患者にのみ治療を行うこと（patient selection）が鉄則である。神経再生治療は長い時間のかかるものであり、治療をスムーズ

に行う上で、充分な説明に基づく患者選択は何よりも重要なことである。

9．人工神経による新しい治療法の展開

末梢神経外傷の治療の中で人工神経を用いて神経を再生させると、神経因性疼痛が劇的に改善する症例がある[10),11)]。また口腔外科、歯科領域では舌神経の再建にも応用が進められている[12)]。この他、耳鼻科、頭頚部外科へも応用され、これまで治療できなかった症例でも機能回復がみられることが判明してきた[13)]。

人工神経の臨床応用に関しては、まず従来の治療法では治せない症例を対象に使用が開始された。このうちカウザルギーに代表される外傷後の神経因性疼痛は、従来のあらゆる治療に反応しないものも多く、外科的なアプローチは禁忌とされてきた。この新しい人工神経による治療は、こういった闇を切り拓く新しい治療法として、疼痛に苦しむ患者の大きな福音になるものと期待が高まっている。

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ORIGINAL ARTICLE

An early mastoid cavity epithelialization technique using a postauricular pedicle periosteal flap for canal wall-down tympanomastoidectomy

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Abstract

Conclusions: Most ears that were treated with a new surgical method 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. *Objectives:* Canal wall-down tympanomastoidectomy was a well established procedure for severe chronic otitis media, especially cholesteatoma. However, this procedure has some defects, so-called cavity problems, caused by non-epithelialized bony wall. The aim of this study was to evaluate the early epithelialized technique for the surface of widely formed external acoustic meatus after canal wall-down tympanomastoidectomy. *Methods:* Twenty-five patients who had been diagnosed with cholesteatoma were divided into two groups. Group I consisted of 15 patients who 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. As a control, 10 patients in group II underwent the standard operation that uses only free temporal fascia grafts. *Results:* A comparison of the two groups showed that it took only 1 month on average for the entire surface of the external auditory meatus of the patients in group I to epithelialize and dry up perfectly, although the same area in all the patients in group II was not dried up perfectly until over 80 days.

Keywords: *Cavity problems, non-epithelialized bony wall, cholesteatoma, free temporal fascia grafts, postoperative complications*

Introduction

The newly formed external acoustic meatus that results from canal wall-down tympanoplasty is a single cavity that joins the mastoid cavity. The volume of this cavity is much larger than that of the original external acoustic meatus, and it is difficult to completely cover the surface of the newly formed bony wall by free temporal fascia grafts alone. Even if the surface epithelialization of the bony wall is accelerated by postoperative treatments, a bone-exposed area is often observed. Furthermore, the remaining non-epithelialized bony wall is difficult to dry, gets crusty, and is easily infected, which consequently causes the recurrence of otitis media [1,2]. 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.

The aim of this study was to assess the ability of this new operative technique to reduce postoperative complications in canal wall-down tympanoplasty.

Material and methods

Twenty-five patients ranging in age from 16 to 78 years who had been diagnosed with cholesteatoma

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