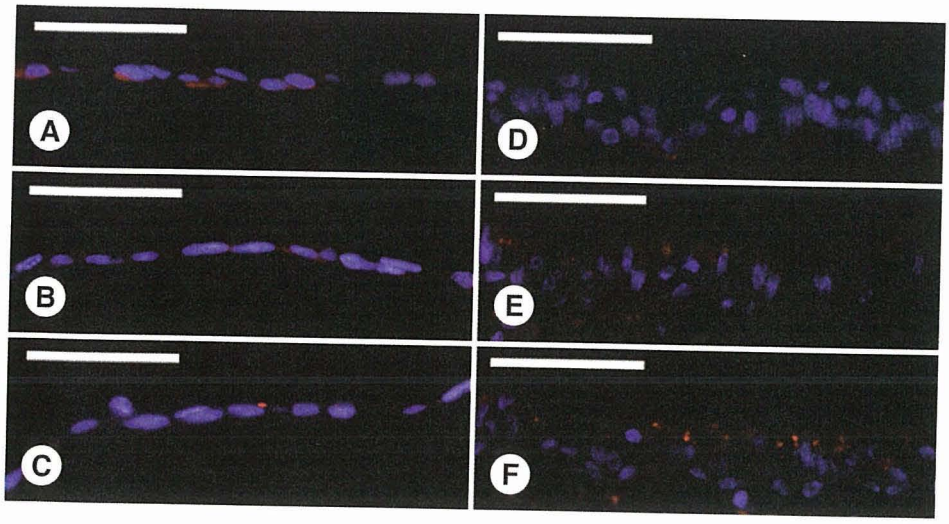

Fig 4. Microscopic images of fluorescent immunostaining of regenerated epithelium and normal tracheal epithelium. Nuclei are blue. Bars — 50.0 μm. **A-C)** Regenerated epithelial cell layers expressing **A)** cytokeratin 14 (red), **B)** cytokeratin 18 (red), and **C)** occludin (red). **D-F)** Normal tracheal epithelia expressing **D)** cytokeratin 14 (red), **E)** cytokeratin 18 (red), and **F)** occludin (red).

tracheal reconstruction in mongrel dogs. In the report, epithelialization began from either end of the grafts and slowly proceeded toward the center, which was the last area to be covered. Because of this pattern, fibrosis occurred in the center of the grafts in association with an acute inflammatory reaction that led to stenosis, resulting in the death of 4 of the 21 dogs. Okumura et al[6] reported a successful animal experiment using an artificial trachea made from spiral polypropylene stent and Marlex mesh woven of polypropylene monofilament as the frame and using collagen extracted from porcine skin for coating the frame as the scaffold for a 2-cm-long circumferential tracheal reconstruction in mongrel dogs. Confluent epithelial coverage in the dogs was confirmed histologically after the dogs were sacrificed 6 months or more after operation. Teramachi et al[1] and Nakamura et al[2] used an artificial trachea that was made from polypropylene stent and Marlex mesh as the frame and used collagenous sponge as the scaffold for carinal replacement in mongrel dogs, and reported successful results at the 5-year observation. Omori et al[3] reported the first human case of using an artificial trachea with a construction the same as used in the previous studies.[1,2] The patient had papillary carcinoma of the thyroid gland with tracheal invasion and underwent hemithyroidectomy, noncircumferential tracheal resection, and reconstruction with a patch of the artificial trachea. Under endoscopic observation, the artificial trachea was seen to be covered with epithelium after 2 months. These results indicate that epithelialization on the lumen of the artificial trachea takes a considerable length of time after reconstruction, and that the larger the replacement with artificial material, the more frequently problems originate from inflammation or excessive fibrosis. It is expected that epithelialization at an early post-reconstruction period helps to prevent problems with grafts. In this study we tried to cover the artificial prosthesis with epithelium by means of tissue engineering and examined its effectiveness for acceleration of epithelialization.

Collagen accounts for about one third of the total protein of an animal and is important not only for supporting tissue construction, but also for differentiation and formation of cells. Collagenous matrix has enabled

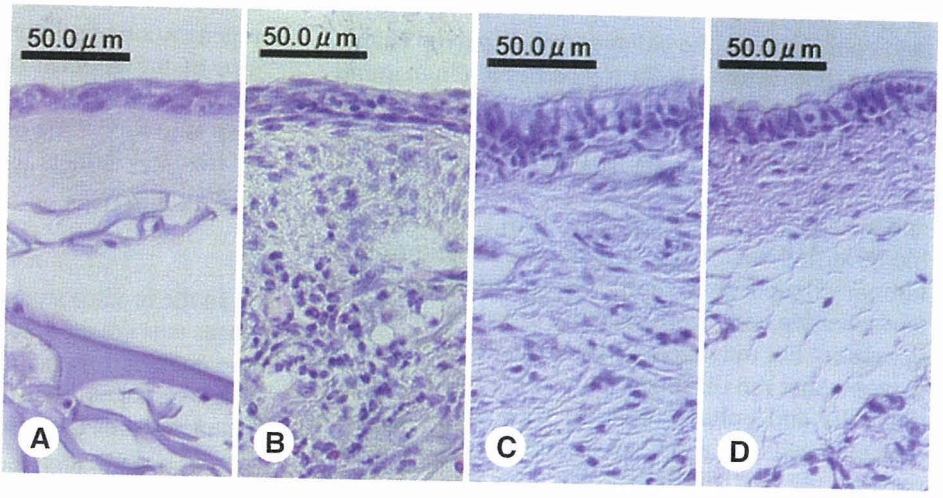

Fig 5. Microscopic images of hematoxylin-eosin staining of defects of tracheas after operation. **A)** Three days after operation. Double-stratified squamous epithelium is observed on gel. Collagenous gel layer is well preserved. **B)** Seven days after operation. Stratified squamous epithelium and infiltration of cells such as fibroblasts, granulocytes, and lymphocytes into gel are observed. **C)** Fourteen days after operation. Columnar ciliated epithelium is observed. Most of gel has disappeared, and subepithelial layer has regenerated. **D)** Thirty days after operation. Same as 14 days after operation.

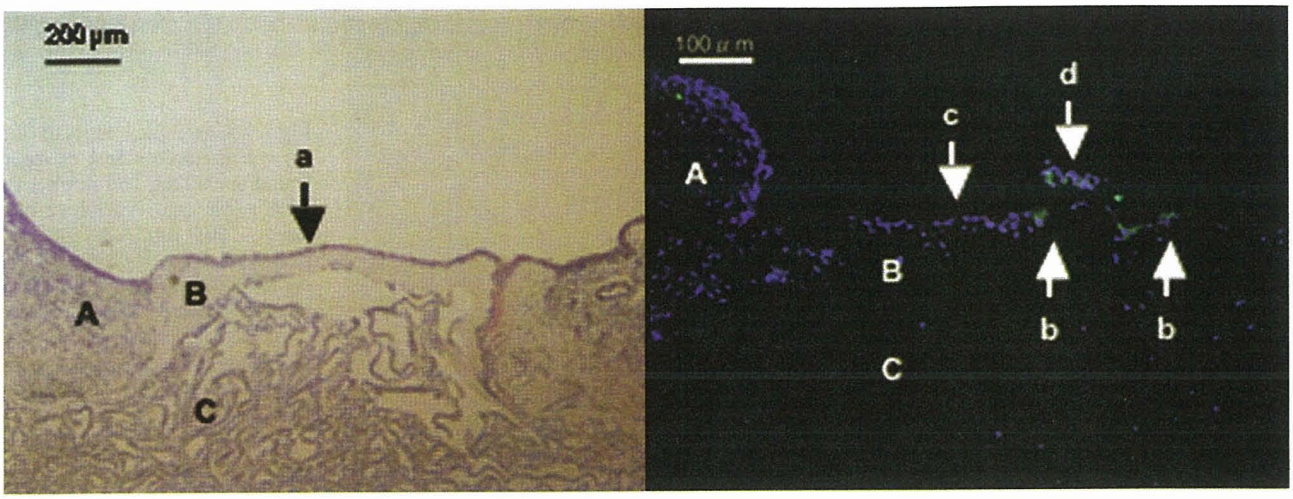

Fig 6. Microscopic images of hematoxylin-eosin staining (left) and fluorescent immunostaining (right) at 3 days after operation. GFP-positive cells (green) are located at central area of regenerated epithelium. Nucleic acid is stained by 4',6-diamidine-2'-phenylindole dihydrochloride and colored blue. A — edge of tracheal defect; B — collagenous gel layer; C — collagenous sponge layer; a — regenerated epithelium; b — epithelium with GFP-positive cells; c — epithelium with GFP-negative cells; d — lump of collapsing gel and GFP-positive epithelium.

many kinds of cells to be cultured in vitro. Cell culture on a collagenous gel — an anchorage-dependent culture called the attached collagen gel culture method — is suitable for epithelial cells. With this method, culture of tracheal epithelial cells of several species, such as rats, guinea pigs, rabbits, dogs, and humans, has been reported.[7-9] Collagen gel can be also stratified on other matrices such as collagen sponge. These two characteristics made collagen gel suitable for this study. In this study, it was confirmed that covering the surface of the prosthesis with epithelium was possible with a collagenous gel layer.

Cytokeratin is found in the cytoskeleton of epithelial cells. Cytokeratin 14 and cytokeratin 18 are markers of epithelial cells. Occludin is a marker of tight junctions that is located on cell-to-cell adhesion in epithelial and endothelial cellular sheets and plays the important role of acting as a barrier to the diffusion of solutes through the intercellular space. In our in vitro study, it was demonstrated that the regenerated epithelium on the collagen gel displayed characteristics that are found in the tracheal epithelium immunohistochemically.

In our in vivo study, the artificial trachea was covered with epithelium through the observation period, and the epithelium differentiated from single- or double-stratified nonciliated squamous epithelium to columnar ciliated epithelium. Green fluorescent protein–positive cells were partially seen at 3 days after operation, but they were not seen from 7 to 30 days. It seems that the disappearance of GFP-positive donor cells and epithelialization with the recipient's epithelial cells occurred simultaneously. One of the reasons for this disappearance may be a kind of allograft rejection. In the in vivo study, infiltration of lymphocytes and granulocytes into the gel was observed at 7 days after operation. Those inflammatory cells probably play a role in immunoreactions against the transplanted graft containing the donor's cells. The allograft rejection can be overcome by using autogenous cells such as the epithelial cells of oral or nasal mucosa, and this is an important subject from the point of view of clinical use. Another reason for the disappearance of GFP-positive donor cells is the weakness of the fixation between the epithelial cells and the collagenous gel. Air currents or the alternately changing pressure with respiration may exfoliate the epithelial layer. It is necessary to find a way to fix the epithelial layer on the collagenous gel more firmly.

In this study, it was confirmed that the donor's epithelial cells with artificial trachea survived until the recipient's epithelial cells formed the epithelium over the defect, and that collagenous gel did not disturb the regeneration of epithelium or subepithelial tissue. The size of the tracheal defects of rats was too small to evaluate the acceleration of epithelialization. The next step is to repeat the experiment using a larger model of tracheal defects and measure the acceleration of epithelialization.

CONCLUSIONS

In the in vitro study, the surface of the artificial tracheas was covered with a tracheal epithelium by a tissue engineering technique. The epithelium on the artificial trachea kept the same immunohistochemical characteristics as normal tracheal epithelium.

After transplantation of the artificial trachea with

the epithelium to the tracheal defect of the rats, the artificial trachea was covered with regenerated epithelium through the observation period. The donor's epithelial cells survived until the recipient's epithelial cells formed an epithelium over the defect.

We believe that the method used in our experiment is an effective way to regenerate the epithelium on the surface of an artificial trachea. With further experimentation, this method should be suitable for clinical application.

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Tissue Engineering of Small Intestinal Tissue Using Collagen Sponge Scaffolds Seeded with Smooth Muscle Cells

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ABSTRACT

In a previously reported attempt to regenerate small intestine with autologous tissues, collagen scaffolds were used without cell seeding or with autologous mesenchymal stem cell seeding. However the regenerated intestine lacked a smooth muscle layer. To accomplish regeneration of a smooth muscle layer, this present study used collagen scaffolds seeded with the smooth muscle cells (SMC) in a canine model. Autologous SMC were isolated from stomach wall and cultured. Two types of scaffolds were fabricated: in SMC (+), cultured SMCs were mixed with collagen solution and poured into a collagen sponge; and in SMC (−), SMCs were omitted. Both scaffolds were implanted into defects of isolated ileum as a patch graft. Animals were euthanized at 4, 8, and 12 weeks; for the last time point, the ileal loop had been reanastomosed at 8 weeks. At 12 weeks, the SMC (−) group showed a luminal surface covered by a regenerated epithelial cell layer with very short villi; however only a thin smooth muscle layer was observed, representing the muscularis mucosae. In the SMC (+) group, the luminal surface was covered completely by a relatively well-developed epithelial layer with numerous villi. Implanted SMCs were seen in the lamina propria and formed a smooth muscle layer. Thus, we concluded that collagen sponge scaffolds seeded with autologous SMCs have a potential for small intestine regeneration.

INTRODUCTION

SHORT-BOWEL SYNDROME (SBS) is a clinical complication of massive small bowel resection characterized by malabsorption and malnutrition. To varying degrees, patients require total parental nutrition; weaning from such alimentation is slow and depends on the area of bowel surface available.[1] The promising treatment for insufficient absorptive surface would be intestinal transplantation; however this therapy requires a donor of appropriate tissue type as well as long-term immunosuppression.[2]

Recent technological progress in the field of tissue en-

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gineering allows many tissues and organs to be regenerated,[3] and some such advances have been reported for the small intestine. Vacanti's group, using polyglycolic acid (PGA) scaffolds, successfully obtained formation of cystic structures seeded with intestinal organoids harvested from neonatal rats.[4–8] They reported that tissue-engineered small intestine improved recovery after massive small bowel resection in a rat model.[9] Other research groups have used small intestinal submucosa (SIS) as a scaffold for small intestine regeneration in rat,[10,11] rabbit,[12] and canine models.[13] Chen and Badylak found that use of SIS patches produced three regenerated layers–mucosa, smooth muscle, and a serosal covering–in a canine model.[13] Hori *et al.* aimed to regenerate small intestine with material of autologous origin and using collagen sponge as a scaffold,[14] but the regenerated intestine lacked a smooth muscle layer, which is essential for functional peristalsis and maintenance caliber of lumen.[15] In an attempt to regenerate a smooth muscle layer of the intestine, collagen sponge seeded with autologous mesenchymal stem cells was used, but any smooth muscle layer still did not regenerate.[16]

Unlike mature skeletal and cardiac myocytes, mature smooth muscle cells (SMC) retain the developmental potential to dedifferentiate, both *in vivo* and *in vitro*.[17] Tissue engineering of urinary bladder or blood vessels using scaffolds seeded with SMCs from bladder or artery were reported to develop an orderly smooth muscle layer.[18–22] SMCs from a given organ show behavior distinct from that of most other organs, particularly in terms of functional responses to various stimuli.[23] It is likely that SMCs from the gastrointestinal tract would perform better for regeneration of intestine. In the present study, we isolated SMCs from the stomach wall, which contains abundant SMCs, to investigate the effect of autologous SMC seeded onto collagen scaffolds for regeneration of small intestine compared to collagen scaffolds without cell seeding in a canine model.

MATERIALS AND METHODS

Animals and anesthesia

Female beagle dogs (under 2 years and weighing 9 ~ 11 kg) were premedicated by intramuscular administration of atropine sulfate at 0.05 mg/kg. Dogs were then anesthetized with 15 mg/kg ketamine hydrochloride and 3 mg/kg xylazine hydrochloride and incubated endotracheally. Halothane and nitrous oxide gas were used for the maintenance of anesthesia during the procedure, under mechanical ventilation. Animal care, housing, and surgery followed the Rules and Regulations of the Committee for Animal Research of the Kyoto Prefectural University of Medicine in Japan.

Isolation and culture of SMCs

Gastric SMCs were harvested from anesthetized dogs by laparotomy. A portion of smooth muscle layer was resected from the anterior wall of the stomach (1 × 1 cm), using 4-0 silk sutures to close the defect of this smooth muscle layer. Postoperatively, animals were maintained on a liquid diet and water for 24 h, followed by resumption of a full diet of dog chow.

Two techniques have already been reported for SMC isolation–explant techniques and enzymatic dispersion. In this study, SMCs were obtained by enzymatic dispersion, because the explant techniques yield a mixture of SMC and myofibroblasts.[20] Enzymatically dissociated stomach wall and SMCs were cultured according to the modified method of Kiwamoto *et al.*[24] In brief, the resected smooth muscle layer was incubated with 0.25% trypsin for 30 min at 37°C and then minced with scissors, followed by incubation with 0.1% collagenase I for 2 h at 37°C and passed through a nylon cell strainer (200 μm). Then, the cell suspension was centrifuged at 1000 rpm for 10 min. The pellets were resuspended in Dulbecco's modified Eagle's medium (DMEM), containing 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 0.1 mg/mL streptomycin (all from Invitrogen, Carlsbad, CA). The suspension was placed in 100 mm dishes coated with collagen type I (Asahi Techno Glass, Chiba, Japan). Cells were grown at 37°C in an incubator with a humidified atmosphere of 5% CO_2. Culture media were changed every 2–3 days. When cultures reached about 80% confluence, cells were resuspended by dissociating them with 0.25% trypsin and 0.2% EDTA (Invitrogen). They were maintained and grown in the same culture medium.

Collagen sponge scaffolds

In this study, we used the collagen sponge Pelnac (Gunze, Kyoto, Japan), which has been used clinically as artificial skin with excellent results in regeneration of the dermis.[25,26] We therefore considered this collagen sponge well suited for promoting regeneration of the smooth muscle layer. Pelnac is composed of a silicone sheet and a sheet of collagen sponge. We used only the collagen sponge sheet, which is 3 mm thick, with a pore size of 70–110 μm and pore volume fraction of 80–95%. Tensile strength of this collagen sponge is 1.4 ± 0.23 (mean $\pm$ SD $\times 10^4$ N/m^2).

Labeling of cells and seeding on scaffolds

SMCs were labeled with chloromethy ibenzamido (Cell Tracker CM-DiI, Invitrogen) at a concentration of 1 μg CM-DiI per 1 mL of Hank's balanced salt solution (Invitrogen). DiI-labeled SMCs were detected using a fluorescence microscope (IX70, Olympus, Tokyo, Japan). SMCs were cultured in the collagen sponge as follows.

Seven volumes of acid-soluble type I collagen solution mixed with 2 volumes of a 5-fold concentration of DMEM and 1 volume of reconstruction buffer (all from Nitta Gelatin, Osaka, Japan). This mixture was kept on ice, mixed with cultured cells at a density of 5×10^6 cell/cm^3, and poured into the collagen sponge. The mixture was incubated at 37°C for 1 h to allow trapping of cells in the collagen sponge. There were collagen sponges with collagen solution containing SMCs (+) or not containing SMCs (−). The collagen scaffolds were cultured for 12 h using DMEM containing 10% FBS before implantation, as described below.

Implantation procedure

After laparotomy, two ileal loops were isolated, together with several feeding and draining vessels, while the interrupted bowel was reanastomosed using a two-layer technique with 4-0 Vicryl (Ethicon, Mountain View, CA) and 4-0 silk to maintain a normally functioning alimentary canal. We created defects (1 × 1 cm) in the small intestine at the middle of the isolated ileal loops and patched these with silicone sheets using 6-0 Vicryl sutures. The silicone sheets were necessary to protect the scaffolds from infection and digestion. SMC (+) and (−) scaffolds were placed below the silicone sheet. They were fixed in place with 6-0 Vicryl sutures (Fig. 1A) and covered on the serosal aspect with omentum. The ileal loops were used to construct a double ileostomy on both sides of the abdominal incision (Fig. 1B). The abdominal incision was closed in two layers with 1-0 silk.

Two animals were euthanized at 4 weeks after implantation, and another two at 8 weeks. The last four animals were divided into two subgroups of two dogs each. At 8 weeks, one subgroup underwent reanastomosis of the SMC (+) ileal loop, and the other subgroup underwent reanastomosis of the SMC (−) ileal loop, with the aim of preventing disuse atrophy (Fig. 1C). Dogs in both subgroups were killed at 12 weeks after implantation. Specimens were examined histologically. Postoperatively, animals were maintained on a liquid diet and water for 48 h and then returned to a full diet of dog chow. An antibiotic (cefazolin sodium, 50 mg/kg/day, i.m.) was given for 5 days.

Histologic, immunohistochemical, and immunofluorescence analyses

Samples of the isolated cells were fixed for 10 min in acetone. Collagen sponge scaffolds before implantation, and also scaffolds harvested later with tissues, were fixed with 10% buffered formalin, processed for embedding in paraffin, and sectioned at a thickness of 4 μm. These scaffolds and tissues were stained with hematoxylin and eosin. Tissues and cells were stained immunohistochemically with an Envision+/HRP detection system (Dako, Kyoto, Japan). In brief, endogenous peroxidase activity was blocked with peroxidase blocking reagent (Dako) for 5 min. Then sections or fixed cells were incubated with mouse monoclonal antibodies against α-smooth muscle actin (α-SMA), basic calponin, vimentin, desmin, von Willebrand factor (vWF), or cytokeratin MNF116 (all from Dako) for 1 h at room temperature. Then the sections or cells were incubated for 30 min at room temperature with peroxidase-labeled polymer conjugated to goat anti-rabbit or goat anti-mouse immunoglobulin. They were incubated with diaminobenzidine (DAB) chromogen for 5 min, counterstained with H&E, and mounted with a cover slip. Between the steps, sections were rinsed gently with Tris-buffered saline. Control sections were incubated in the absence of primary antibody.

In the immunofluorescence study, in order to examine the location of DiI-labeled SMCs, paraffin-embedded tis-

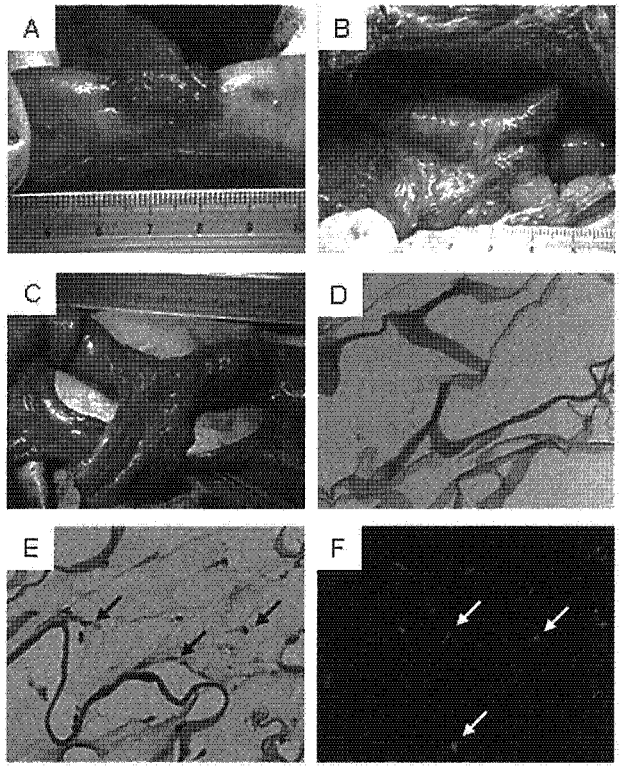

FIG. 1. Surgical technique and collagen scaffolds for SMC (+) and SMC (−) groups. **(A)** SMC (+) or SMC (−) scaffolds were implanted as patch grafts into defects created in two isolated ileal loops. **(B)** The patch graft was covered with omentum, and the two ileal loops were used to construct a double ileostomy on the anterior abdominal wall bilaterally. **(C)** At 8 weeks after implantation, the ileal loops were reanastomosed to avoid disuse atrophy. **(D and E)** Hematoxylin and eosin staining of SMC (−) **(D)** and SMC (+) scaffolds **(E)**. SMC were seeded in the lattice spaces of the collagen sponge in the SMC (+) scaffolds (arrows). **(F)** Most cells of the SMC (+) scaffolds are labeled with DiI (arrows). **(D–F)** Original magnification × 100.

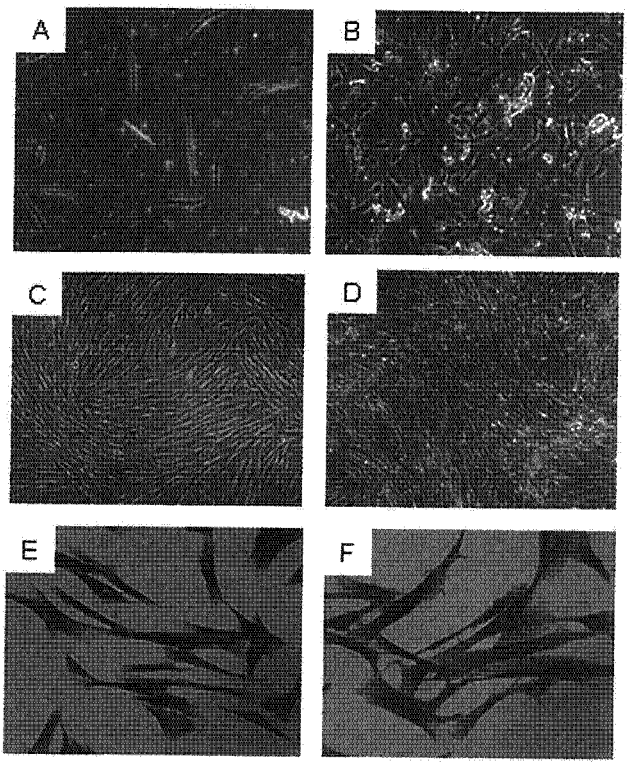

FIG. 2. Isolated and cultured SMCs. **(A)** Immediately after enzymatic isolation, SMC were characterized by a slender shape. **(B)** At day 3 in culture, SMCs show a broad, spreading shape with several mitotic figures. **(C)** At day 7, the cultured SMCs appear subconfluent and have a fusiform shape with groups of cells aligned closely, in parallel orientation. **(D)** At day 12, the cultured SMCs show a hill-and-valley pattern with numerous areas of cellular retraction. The SMC express α-smooth muscle actin **(E)** and basic calponin **(F)**. Original magnification × 40 **(A–D)**; × 100 **(E and F)**.

sue samples were cleared by washing with xylene, followed by air drying at room temperature for 5 min. The secondary antibody was fluorescein isothiocyanate (FITC) conjugated to goat anti-rabbit or goat anti-mouse immunoglobulin (Dako); after its application, slides were washed and specimens were mounted with cover slips using Vectashield mounting medium containing 4′,6-diamidino-2-phenylidole (DAPI, Dako). Specimens were examined using a fluorescence microscope (IX70, Olympus).

Electron microscopy

Smooth muscle cells that were only seeded in the collagen sponge and implanted at SMC 12 weeks were examined by transmission electron microscopy (TEM). Specimens were cut into 1-mm cubes and fixed in 2% glutaraldehyde in cacodylate buffer for 12 h at room temperature. The specimens were further fixed in osmium tetroxide, dehydrated in graded alcohol, and embedded in epoxy resin. Ultrathin sections were stained with lead acetate and lead citrate. The specimens were observed using a transmission electron microscope (JEM-2000EX, JEOL, Tokyo, Japan).

RESULTS

Smooth muscle cells from stomach wall

Smooth muscle cells obtained from the stomach wall by enzymatic dispersion initially were characterized by a slender shape (Fig. 2A). After culture for 3 days, SMCs began to redifferentiate, and numerous cells undergoing mitosis were observed (Fig. 2B). At 7 days, SMCs appeared subconfluent, with individual regions of cells aligned in close, parallel orientation (Fig. 2D). At 12 days, SMCs showed morphologic characteristics typical of smooth muscle cells, as indicated by a hills-and-valleys appearance described by Chamley-Campbell *et al.*[27] (Fig. 2D). SMC identity was confirmed by immunoreactivity for α-SMA and for basic calponin (Fig. 2E and F). Cultured SMCs did not stain for an epithelial marker (cytokeratin) or an endothelial marker (vWF). In addition, all cells were stained for vimentin while very few cells were stained for desmin (data not shown).

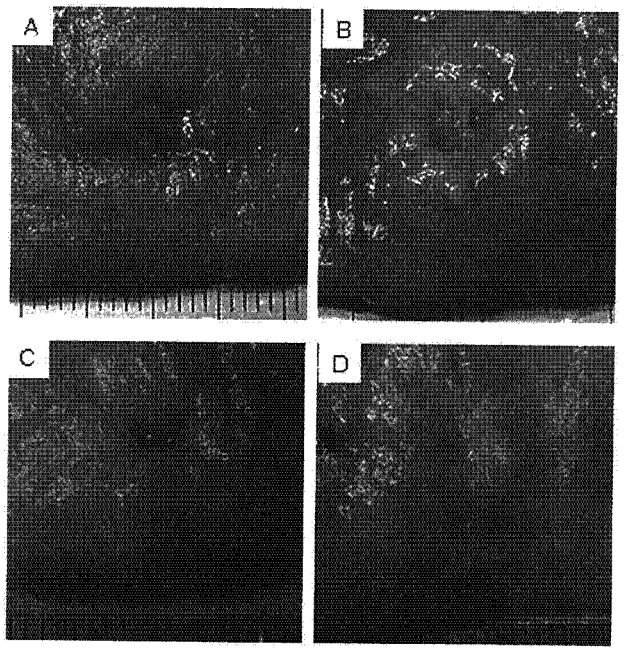

FIG. 3. Macroscopic findings on the luminal side of the graft area. The SMC (−) group **(A)** and SMC (+) group **(B)** at 4 weeks after implantation, and the SMC (−) group **(C)** and SMC (+) group **(D)** at 12 weeks after implantation. **(A and B)** In both groups, the luminal surface of the graft area was not covered with mucosa at 4 weeks, but had an ulcerative appearance. **(C)** At 12 weeks, the graft surfaces in the SMC (−) group were covered by regenerated mucosa that was depressed relative to the adjacent mucosa. **(D)** At 12 weeks in the SMC (+) group, it was difficult to macroscopically distinguish the appearance and contour of the regenerated mucosa from that of the normal mucosa.

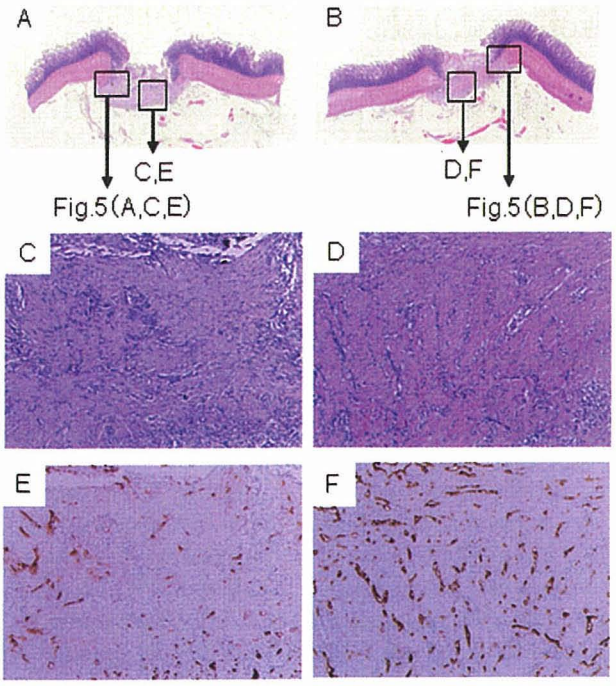

FIG. 4. Histologic and immunohistochemical features of the graft area at 4 weeks after implantation. H&E staining is shown for the SMC (−) group **(A)** and SMC (+) group **(B)**. **(C–F)** High-power views of boxed regions in **A** and **B:** H&E staining **(C and D)** and immunostaining for von Willebrand factor **(E and F)**. A considerably greater number of blood vessels are observed in the graft area in the SMC (+) group than in the SMC (−) group. Original magnification × 2 **(A and B);** × 100 **(C–F).**

Collagen sponge scaffolds seeded with smooth muscle cells

The scaffolds had shrunk about 10% from their original size at 12 h after seeding. SMCs were seeded on lattice spaces of collagen sponge scaffolds; almost all cells showed DiI labeling (Fig. 1E and F). When we seeded the cells without collagen solution, far fewer cells remained in the sponge scaffolds because they passed through the pores of the sponge. In addition, the seeded cells were distributed unevenly in the collagen sponge when collagen solution was not used for seeding (data not shown).

Macroscopic examination

All animals survived until the scheduled time for euthanasia. During the experiment, change in body weight did not exceed 1 kg, and no anastomotic problems, such as dehiscence, occurred. The serosal aspect of both scaffolds remained firmly covered with omentum after 4, 8, and 12 weeks. At 4 weeks after implantation, Vicryl sutures had nearly disintegrated, and silicone sheets had almost come off the luminal surface of the graft site. With both groups, the luminal surface of the graft area was not covered with mucosa at 4 weeks but rather had an ulcer-like appearance (Fig. 3A and B). At 8 weeks, graft surfaces were covered with regenerated mucosa that was depressed relative to adjacent mucosa. At 12 weeks, the SMC (−) group showed the same depressed contour (Fig. 3C). At 12 weeks in the SMC (+) group, however, the appearance and contour of regenerated mucosa were difficult to distinguish macroscopically from normal mucosa (Fig. 3D).

Microscopic examination

Postoperative interval of 4 weeks

SMC (−). The graft site was infiltrated with cells, capillaries, and connective tissue. Immunohistochemical staining for vWF indicated the location of blood vessels,

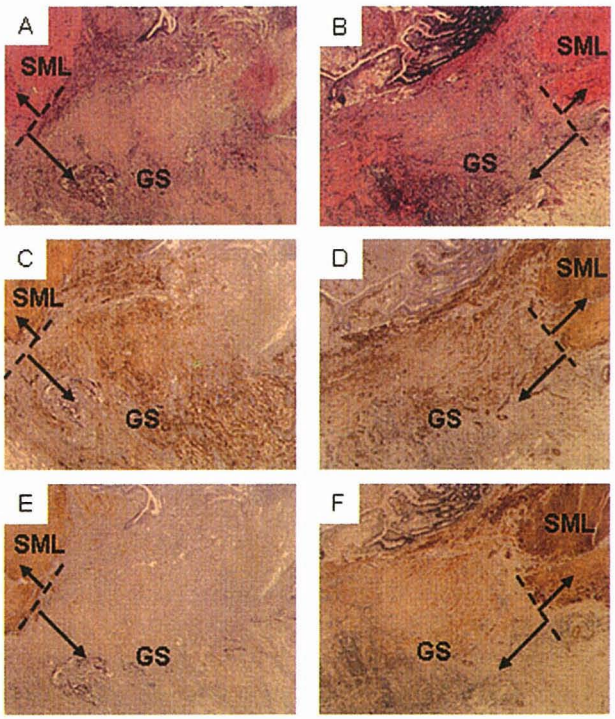

FIG. 5. Histologic and immunohistochemical features of the graft area at 4 weeks after implantation. **(A, C, and E)** High-power views of boxed regions in **Fig. 4 A**. **(B, D, and F)** High-power views of boxed regions in **Fig. 4 B.** **(A and B)** Hematoxylin and eosin staining; **(C and D)** Immunostaining for α-SMA; **(E** and **F)** immunostaining for basic calponin. To the left of the dashed line is original smooth muscle layer (SML); to the right is the graft site (GS) in **A, C, and E.** To the right of the dashed line is SML; on the left is GS in **B, D, and F**. In the SMC (−) group, most cells at the graft site were myofibroblasts because they were stained immunohistochemically for α-SMA but not for basic calponin **(C and E)**. In the SMC (+) group, both SMC and myofibroblasts were stained immunohistochemically with anti-α-SMA **(D)**. SMC were identified by their immunoreactivity with anti-basic calponin. SMC were sparsely dispersed among the more numerous myofibroblasts **(F)**. Original magnification × 2 **(A and B);** × 40 **(C–H).**

some of which were in the graft area (Fig. 4C and E). Most cells at the graft site were myofibroblasts, as they stained immunohistochemically for α-SMA but not basic calponin[29] (Fig. 5A, C, and E). As noted macroscopically, the luminal surface was not yet covered with epithelial cells.

SMC (+). The graft site was infiltrated by cells, capillaries, and connective tissue. Immunohistochemical staining for vWF showed that the SMC (+) group had a considerably higher number of blood vessels in the graft site than in the SMC (−) group (Fig. 4C and F). Both SMCs and myofibroblasts were stained immunohistochemically with anti-α-SMA. SMCs were identified by their immunoreactivity with anti-basic calponin. SMCs were sparsely dispersed among the more numerous myofibroblasts. These cells showed DiI labeling by fluorescence microscopy, confirming that their origin was of implanted cells (Fig. 5B, D, and F). A transitional mucosal epithelial layer began to line the luminal surface of the graft site at the end of the anastomosis.

Postoperative interval of 8 weeks

SMC (−). The luminal surface of the graft site was covered with a monolayer of mucosal columnar epithelial cells. Collagen sponge scaffolds were absorbed, and myofibroblasts had disappeared. A thin smooth muscle layer, the muscularis mucosae, was stained immunohistochemically with anti-α-SMA and anti-basic calponin (Fig. 6 A, C, and E).

SMC (+). The luminal surface of the graft site was covered with a regenerated epithelial cell layer that included goblet cells and had a villus-like configuration, although these villi were shorter than those in adjacent normal mucosa. Immunohistochemical staining with anti-α-SMA and basic calponin and detection of the labeled cells using a fluorescence microscope showed the presence of implanted SMCs in the lamina propria and formed a deeper smooth muscle layer (Fig. 6B, D, and F). The collagen sponge scaffolds were absorbed, and myofibroblasts had disappeared. Implanted SMCs were multilayered, and the surface area of the graft site shrank.

Postoperative interval of 12 weeks (reanastomosed at 8 weeks)

SMC (−). The luminal surface of the graft site was covered with a regenerated epithelial cell layer showing very short villi. No significant change in thickness of the muscularis mucosae was noted between 8 and 12 weeks.

SMC (+). The luminal surface of the graft site was covered completely by a relatively well-developed epithelial layer with numerous villi and also an orderly smooth muscle layer (Fig. 7A–C). At this time point, these villi were more developed and the implanted SMCs had more polarity than they had at 8 weeks. To examine the dual location of DiI-labeled SMCs and basic calponin expression at the graft site, samples were stained for immunofluorescence and analysis using a fluorescence microscope. Implanted SMC were noticed to organize into a circumferential smooth muscle layer and were present in part of lamina propria (Fig. 7D–I).

Phenotypic change of SMC

SMC underwent a spontaneous change in phenotype–from the contractile state to the synthetic state–during the culture.[30] SMCs seeded only in the collagen sponge contained large nuclei, large amounts of rough endoplasmic reticulum and mitochondria, and very few myofilament bundles with dense bodies (Fig. 8A). These findings show that these SMCs are in the synthetic state.[30] SMCs implanted at 12 weeks contained many bundles of thick and thin myofilaments with associated dense bodies and organelles, such as mitochondria, a Golgi appa-

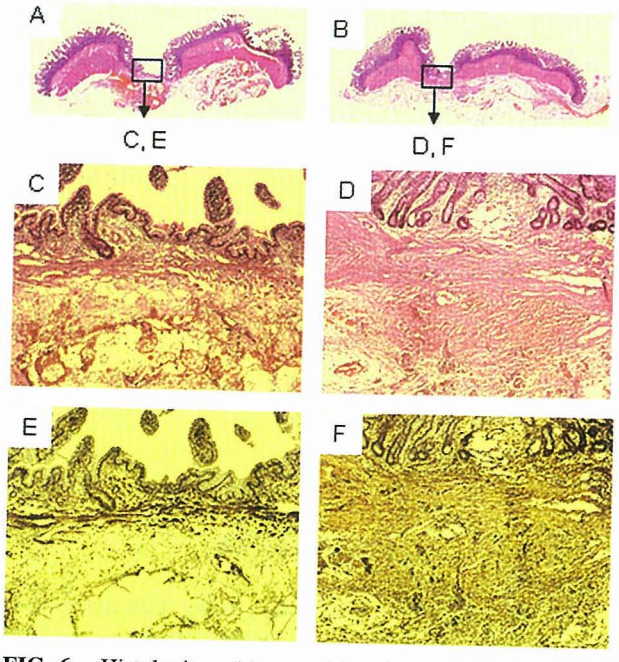

FIG. 6. Histologic and immunohistochemical features of the graft area at 8 weeks after implantation. Hematoxylin and eosin staining is shown for the SMC (−) group **(A)** and SMC (+) group **(B)**. **(C–F)** High-power views of **A** and **B**: H&E staining **(C and D)**; immunostaining for basic calponin **(E and F).** The luminal surface of the graft site was covered with a monolayer of mucosal columnar epithelial cells **(C)**. A thin smooth muscle layer, the muscularis mucosae, was stained immunohistochemically with anti-basic calponin **(E)**. The luminal surface of the graft site was covered with a regenerated epithelial cell layer that included goblet cells and had a villus-like configuration; however, these villi were shorter than those in adjacent normal mucosa **(D)**. Implanted SMCs formed a thicker smooth muscle layer **(F)**. Original magnification × 2 **(A and B);** × 100 **(C–F).**

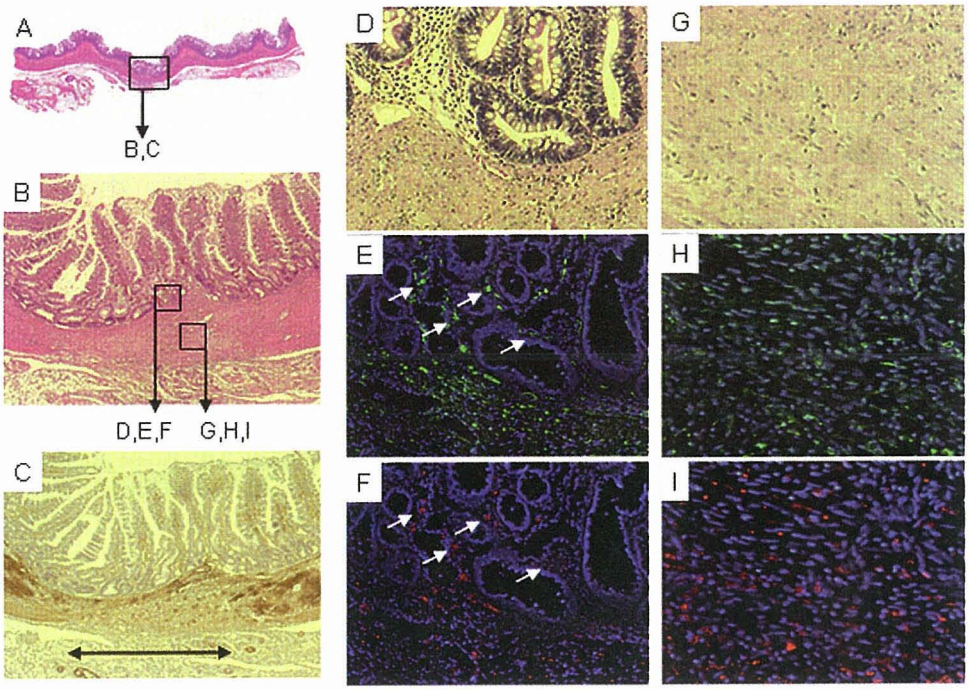

FIG. 7. Histologic, immunohistochemical, and immunofluorescence features of the graft area at 12 weeks after SMC (+) scaffold implantation. **(A)** Hematoxylin and eosin staining. **(B and C)** High-power views of **A:** H&E staining **(B);** immunohistochemical staining for basic calponin **(C).** The graft site is shown by the bar. **(D–I)** High-power views of **B:** a portion of the lamina propria of the graft site**(D–F)**; a portion of the smooth muscle layer of the graft site**(G–I).** **(D and G)** H&E staining; **(E and H)** immunofluorescence staining for basic calponin. **(F and I)** The location of SMC labeled by DiI. The luminal surface of the graft site was completely covered by a relatively well-developed epithelial layer with numerous villi and an orderly smooth muscle layer. It was observed that implanted SMCs were organized into a circumferential smooth muscle layer and were present in a part of the lamina propria (arrows, **E and F**). Original magnification × 2 **(A);** × 40 **(B and C);** × 400 **(D–I).**

ratus, and ribosomes that are localized in the perinuclear region. These findings indicate that these SMCs are in the contractile state (Fig. 8B).[30]

DISCUSSION

We pursued organ tissue engineering with autologous cells in order to avoid transplantation problems such as rejection, long-term immunosuppression, donor scarcity, ethical issues, and infection. This study focused on regeneration of the smooth muscle layer of the intestine, using collagen scaffolds seeded with autologous SMCs suspended in collagen solution. Although tissue engineering of bladder and vessels using SMC-seeded scaffolds had been reported previously,[18–21] this study may be the first demonstration of tissue engineering of intestine using SMC-seeded scaffolds.

We obtained SMCs from the smooth muscle layer of the stomach wall, which has an abundance of smooth muscle. We were able to resect a portion of the smooth muscle layer of stomach wall via an incision of <3 cm in length, with an operative time of <15 min and without any complications. Therefore, this procedure seems to be safe and minimally invasive. Thus, we considered the stomach wall to be an appropriate source of SMCs. Our simple method of enzymatic dispersion made it possible to obtain differentiated SMCs of high purity. Isolated SMCs entered mitosis and proliferated *in vitro* in 10% blood serum. Our results were similar to those of previous reports:[18–21,23] when we seeded SMCs on the collagen sponges without collagen solution, many cell-free spaces were seen on the collagen sponge, as was reported in a study with poly (lactic-co-glycolic acid) (PLGA) mesh-collagen hybrid scaffolds.[22] Transmission electron microscopic analyses revealed that SMCs that were only seeded in the collagen sponge were in a synthetic state. On the other hand, implanted SMCs changed their phenotype from the synthetic to the contractile state *in vivo*.

At an early time point in our study (4 weeks after implantation), the graft site was infiltrated by numerous myofibroblasts in both groups. In the SMC (+) group, the implanted SMC were sparsely distributed among many myofibroblasts in the graft site. The myofibroblasts existing during wound healing were reported to disappear

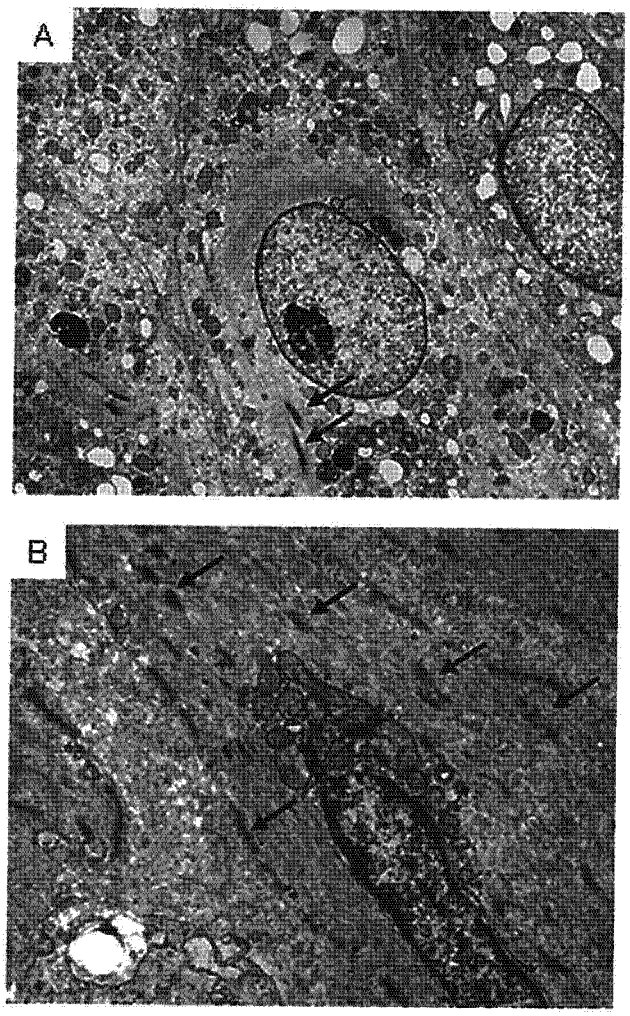

FIG. 8. Transmission electron microscopic appearance of SMCs. SMC just seeded into the collagen sponge shows large nuclei and a large amount of rough endoplasmic reticulum and mitochondria and very few myofilament bundles with dense body (arrows). This cell is in the synthetic state **(A)**. SMC implanted at 12 weeks contains several bundles of thick and thin myofilaments with associated dense bodies (arrows) and organelles, such as mitochondria, a Golgi apparatus, and ribosomes that are localized in the perinuclear region. This cell is in the contractile state **(B)**. Original magnification × 3000 **(A)**; × 12,000 **(B)**.

gradually later.[29] At 8 weeks in the SMC (−) group, no myofibroblasts remained and only a thin smooth muscle layer (muscularis mucosae) was seen. In the SMC (+) group at this time point, myofibroblasts had disappeared and implanted SMCs were forming a smooth muscle layer, although their arrangement within the layer was random. At 12 weeks after implantation in the SMC (+) group, implanted SMCs demonstrated polarity of arrangement, appearing as the circular muscle layer without any evidence of longitudinal muscle layer formation. In the future we plan to investigate the character and function of this regenerated smooth muscle layer.

Angiogenesis in the submucosa supports the healing of the overlying epithelium. Immunohistochemical staining for vWF indicated the location of blood vessels. We included the picture of immunohistochemical staining for vWF in the SMC (+) and SMC (−) groups at 4 weeks after implantation. A considerably greater number of blood vessels was observed in the graft area in the SMC (+) group than in the SMC (−) group. These findings suggest that implanted SMC may release some angiogenic growth factors, such as vascular endothelial growth factor and basic fibroblast growth factor. As a result, implanted SMCs may accelerate mucosal healing. The lamina propria, which forms the cores of villi, consists of connective tissue fiber, fibroblasts, mature fibrocytes, and smooth muscle cells.[32] Our implanted SMCs were also seen in the lamina propria. In this study, SMC (+) group showed a relatively well-developed epithelial layer with numerous villi, while the SMC (−) group had a regenerated epithelial cell layer with very short villi. Recent studies have demonstrated that intestinal fibroblasts promote proliferation of intestinal epithelial cells *in vitro*.[33,34] These results suggest that the lamina propria has an important role in growth of mucosa and formation of villi. We believe that growth of the lamina propria was promoted by SMC implantation, resulting in a more normal appearance of the regenerated mucosa.

Graft sites shrank to a smaller area than the original size of scaffolds. For regeneration of small intestine in a tubular configuration, it is necessary to prevent the shrinkage of the graft area. Shrinkage may occur due to the following reasons. First, the mechanical strength of this collagen sponge may not have been very low and degradation time of this collagen sponge may have been very short. To regenerate the small intestine tissue, the scaffold may need more long time to keep the structure. Second, the number of seeded SMCs may have been too small. Because implanted SMCs are constituents of both smooth muscle layer and lamina propria, application of large numbers of SMCs to the scaffolds may be needed. Future investigations *in vivo* will determine the optimal number of SMC to be seeded. Third, growth of blood vessels into the scaffolds may have been insufficient, even though neovascularization could be seen in the graft site at 4 weeks. Recent reports have demonstrated the angiogenic effect of vascular endothelial growth factor or hepatocyte growth factor in tissue engineering.[35,36] In the future we will investigate the effect of such tropic factors in our scaffold model.

In this study, regenerative epithelial cells immediately covered the scaffold. However, a much longer time will be required to regenerate mucosa when this

is attempted over a wide area. Culture of intestinal epithelial cells *in vitro* is difficult. Epithelial units containing intestinal stem cells disaggregate soon after plating, and the isolated cells die within a few days.[37] However, a recent study reported success in culturing of intestinal epithelial cells on amniotic membranes or on fibroblasts embedded in collagen gel.[34,37] We plan to investigate the culture of autologous intestinal epithelial cells on a collagen scaffold seeded with SMCs. If this approach succeeds, far more extensive regeneration of intestine over a reasonably short time will be expected.

In conclusion, implanted SMCs formed a smooth muscle layer and possibly accelerated epithelization of the luminal surface. Collagen sponge scaffolds seeded with autologous smooth muscle cells show a potential for regeneration of small intestine.

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甲状腺癌治療における気道の再生医療

Regenerative medicine of airway reconstruction for the treatment of thyroid cancer

Regenerative medicine of airway reconstruction for the treatment of thyroid cancer

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Keywords 甲状腺癌 気道 再生医療 人工気管 スキャフォールド

はじめに

甲状腺癌は悪性腫瘍の中では予後が比較的良好であり，分化癌では10年累積生存率は95％以上と高くほぼ満足する成績が得られているといえるが，喉頭，気管，食道，大血管，縦隔など正中部の隣接臓器へ浸潤した場合は難治である[1]。特に喉頭や気管に浸潤した例では，喉頭機能を温存できるか，気管切開を回避できるかなどがQuality of Life（QOL）に大きく影響する。

気道は鼻腔，咽頭，喉頭，気管と気管支・細気管支によって構成されるが，喉頭，気管，気管支は管状の枠組みを軟骨が保持しており，内腔面は外界に接している。気道に悪性腫瘍が浸潤した例では病変切除後の，気道欠損部の再建が問題になる。これには，気道としての硬度をもった枠組みと内腔粘膜を同時に再建するのが理想的である。従来，硬性組織には各種の軟骨，骨，人工材料などが[2]，内腔面組織には皮膚や粘膜などが用いられたが，二期的にしかも他部位の手術が必要であった。また，気管切除後の端々吻合術は，縫合不全の可能性や術後の挿管および頸部前屈体位など解決すべき問題点が残されている。

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人工気管については1960年代から数多くの研究が行われてきたが[3]，満足な臨床成績を残すことができなかった。1995年，中村らは組織工学的な手法で，自己組織の再生を誘導するようにデザインした新しい人工材料を開発した。本人工材料をスキャフォールド（足場）として用い，動物実験で気管組織の再生を実現できることが報告され[4,5]，筆者らは人工材料を改良し輪状軟骨の切除後の欠損モデルに移植し，正常と同等の硬さをもち，かつ良好な上皮化が得られることを報告した[6]。

2002年，筆者らは本技術を用いて「気道の再生医療」を世界に先駆けて臨床例に行い[7]，現時点では6例に施行しており術後経過は良好である。このうち甲状腺癌の切除後の即時再建は3例である。

本稿では，組織工学的手法による気道再生の基礎技術とその臨床応用例を呈示し，さらに次のステップとして解決すべき課題を示す。

スキャフォールドの開発

自己組織の再生を誘導するようにデザインして人工材料を開発した[1,5]。管状の枠組みを保持するためポリプロピレン製のメッシュを管状にし，これを同質の材料でリング状に補強し，さらに組織再生のスキャフォールドとしてその周囲にコラーゲンスポンジを付

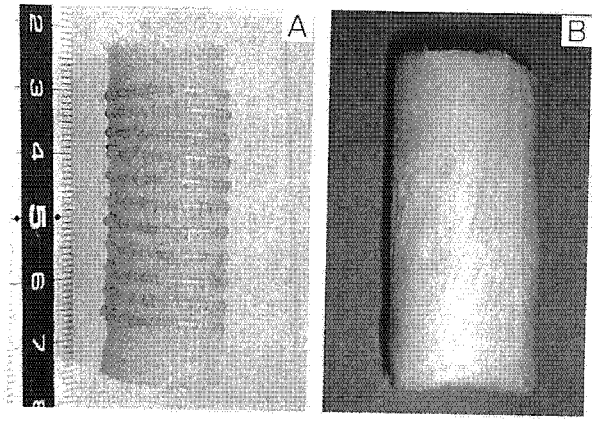

図1　スキャフォールド
A：構造（ポリプロピレンメッシュ管と補強のリング）
B：外観（Aにコラーゲンスポンジを付加）

加した（図1）。ポリプロピレン製メッシュは特定保険材料として従来から胸壁や腹壁の補強を目的に実際に臨床に使用されている材料で，生体に取り込まれるメッシュの最適な編み目の大きさを開発の過程で検証し260μmとした。コラーゲンスポンジは医療用のブタ皮膚由来のⅠ型およびⅢ型コラーゲンを用いた。本人工材料ではコラーゲンとメッシュがはずれにくいようにするために，コラーゲン液をメッシュ上に重層コーティングして厚く付着させ，140℃，24時間の熱架橋を加えた。

動物実験の概要

動物実験は，当該施設の動物実験規定にのっとり，愛護的に動物を扱い疼痛を与えないように全身麻酔で行った。ビーグル犬を用い，頸部気管および胸部気管では，環状切除後に自己組織再生型の人工材料で気管断端を覆うように縫合した[1)5)]。輪状軟骨では，弓部（前半部約1/2周）を切除し，人工材料を欠損範囲に応じて1/2周から2/3周を用いた[6)]。人工材料は自己の血液でコラーゲンを湿潤させてから用いた。術後，気管内視鏡では内腔面は良好な上皮化を認め，少数例で肉芽や内腔狭窄を認めたが，いずれも軽度で呼吸に問題はなかった。組織学的評価では，管状の再生組織管のメッシュ内に結合織が入り込み，再生した気道上皮には電子顕微鏡で線毛を確認した。軟骨組織は再生しなかったが，再生組織の硬度は機械的圧縮試験で正常気管および輪状軟骨と同程度であった。最長5年の観察で，気管の上皮再生は良好で問題なく経過している[1)5)]。

これらのことから，自己組織再生型の人工材料は頭頸部癌の気道浸潤，声門下や気管の炎症性狭窄などにおける気管，輪状軟骨切除後の再建に臨床応用可能と考えられた。

臨床応用

動物実験の結果をふまえ，京都大学医学部倫理委員会，福島県立医科大学医学部倫理委員会の承認を得て，ヘルシンキ宣言にのっとり，患者・家族には治療内容を十分に説明し同意を得た上で，「喉頭・気管の再生治療」のヒトへの応用を行った。

2002年に第1例目として，甲状腺進行癌の気管浸潤例に対して気道の再生医療を行った[7)]。症例は79歳女性，主訴は前頸部腫脹。CTにて，甲状腺右葉全体を占める直径約5 cmの腫瘍を認め，気管内視鏡検査では声門下に続く気管内腔の右側に隆起を認め，甲状腺腫瘍の気管浸潤が疑われた。手術は全身麻酔下に行った。甲状腺右葉は腫瘍で占拠され頸部気管に癒着していたため，安全域を付けて3気管輪，半周にわたって切除した。本人工材料の2/3周分をトリミングし，自己の血液を注入し，その欠損部をパッチする形で縫合した。気管切開孔をおかず一期的に再建し，術当日に抜管した。術後2週間にはコラーゲンがみられ，術後2ヵ月でほぼ上皮化し人工材料は被覆され，術後3年7ヵ月では，気管内腔面は上皮で覆われ組織再生は良好である（図2）。

第2例目は71歳男性，主訴は前頸部腫脹。CTにて，

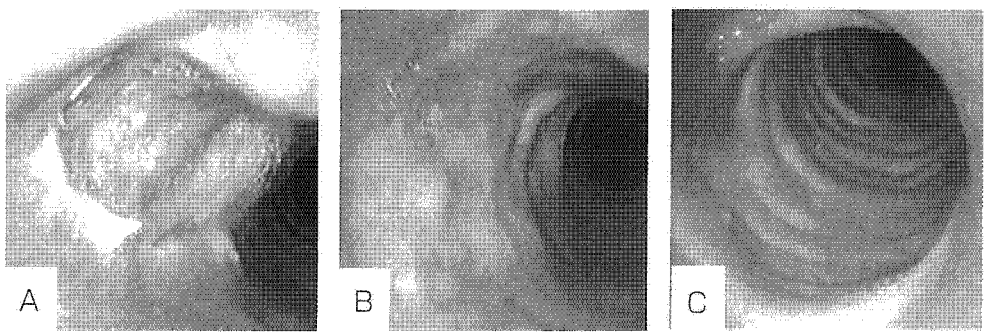

図２　臨床例１：気管浸潤例

A：術後２週間の内視鏡所見
B：術後２ヵ月の内視鏡所見
C：術後３年７ヵ月の内視鏡所見

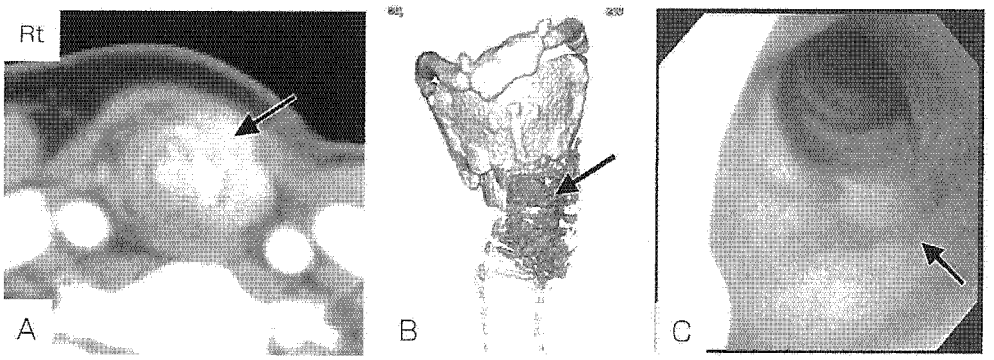

図３　臨床例３：輪状軟骨気管浸潤例

A：術前のCT，輪状軟骨左側への腫瘍浸潤（→）
B：術後の3D-CT，人工材料（→）
C：術後１年の内視鏡所見，上皮化は完了している（→）

甲状腺右葉に腫瘍を認め，甲状腺癌気管浸潤が疑われた。手術は全身麻酔下にて行い，甲状腺腫瘍を露出した。腫瘍は右反回神経を巻き込んでおり，神経切断を余儀なくされた。腫瘍は第１-２気管輪に浸潤しており，約1/3周切断した。その欠損部は10mm×12mmで，本人工材料をトリミングして1/2周分の材料に自己の血液を注入し，気管欠損部をパッチする形で縫合した。術後３日目に軽度のair leakを認めたが，ドレインと圧迫で軽快した。術後２年６ヵ月経過した現在，気管内腔の上皮化は良好で順調な経過である。

第３例目は59歳女性。1988年に他施設にて甲状腺癌の診断にて甲状腺左葉切除術を施行され，2001年に局所再発し甲状腺全摘術を行った上で^{131}I内照射を施行された。2004年に局所再発し，CTにて腫瘍は輪状軟骨，気管への浸潤が認められた（**図３A**）。

術中所見は，輪状軟骨弓部および第１-２気管軟骨の正中から左半分が破壊されており，腫瘍が露出していた。左反回神経は腫瘍を貫いており，切断を余儀なくされた。腫瘍を鋭的に切除し，切除断端を迅速病理診断に提出し陰性を確認して切除を終了した。輪状軟骨弓部と第１-２気管輪の半周を切除した。気道欠損部に本人工材料をトリミングして2/3周分の材料に自

己の血液を注入し，パッチする形で縫合した。術後の3D-CT像（図3 B）から移植した人工材料の大きさがわかる。最後に気管孔を閉鎖して手術を終了した。

術直後の喉頭内視鏡検査では両披裂部の浮腫を認めた。輪状軟骨の広範な切除により術後の喉頭浮腫の持続が予想されたため，一晩挿管したままで経過観察し，翌日に内視鏡で喉頭浮腫の軽減を確認した上で抜管した。再建術後2ヵ月で再建材料の内腔は薄く被覆されたが，内腔が完全に上皮化するには約10ヵ月を要した。図3 Cに術後1年の内視鏡像を示す。術後1年4ヵ月では，肉芽や再狭窄はなく特に問題を生じていない。

考　察

臓器再生にはスキャフォールド，細胞，環境調節因子が必要で，この三要素に加えて血流が供給されると臓器再生が得られるとされている。我々は自己組織の再生を誘導するようにデザインしたスキャフォールドを移植することで，気道の組織再生を図った。

組織工学（tissue engineering）は，工学的手法を使って細胞を二次元的，三次元的に組み上げ，本物の臓器や組織に近いものを再生させようというもので，Vacanti，Langerらによって始められた。彼らのtissue engineeringは，体外で細胞を培養して目的とする組織を作り，これを体内に移植する方法である[8]。

幹細胞や前駆細胞を移植することで組織再生を図ろうという研究が数多く行われている。自己由来の細胞移植としては，循環器領域で下肢や心筋の血管再生を目指した血管内皮前駆細胞の移植[9]，整形外科領域で骨関節疾患の治療に骨髄間葉系幹細胞の移植[10]，眼科領域で角膜再生を目指して角膜上皮幹細胞や口腔粘膜細胞を羊膜上や温度応答シート上で培養した移植などがある[11][12]。

一方，我々の研究グループでは，体内の，再生を目的とする臓器の場所で組織を再生させる「*in situ* tissue engineering」という新しい概念に基づいて，1995年以後，動物実験で自己組織再生型の人工材料を移植し気管，小腸，胃，輪状軟骨などが再生することを報告してきた[1)-6)13)14)]。これらの実験では細胞移植や増殖因子は使わずにスキャフォールドの移植のみでの組織再生を行ってきた。Vacantiらのように体外で組織を再生してから移植する方法や，自己由来であっても細胞移植を伴うと，生きた細胞や組織を取り扱うので感染症対策や細胞の品質管理など臨床応用へのハードルが高い。これらの方法に比べて，我々の行っている*in situ* tissue engineeringによりスキャフォールドのみを移植する手法は，臨床応用に近いといえる。

甲状腺癌による正中部の隣接臓器浸潤において，気道は上方では輪状軟骨，披裂軟骨，甲状軟骨，声帯，下方では頸部気管から胸部気管をどう取り扱うかが重要課題であり，安全域をどの程度とるかが問題となる。進行甲状腺癌の喉頭・気管浸潤例では，喉頭温存を図りながら鋭的治癒切除と機能的再建を積極的に行うことで，呼吸，嚥下，発声の機能障害を生じることなくQOLを維持して，治療成績の向上が期待される。現在，本再生治療は頸部気管・輪状軟骨の部分欠損に対する再建法としては，手技が容易であること，気管切開を回避できること，再建材料のサイズをあまり考慮しなくても切除範囲を決定できるなど，有望な治療法といえる[7)]。

一方，臨床応用の経過から被覆した人工材料内腔面の上皮化には最低でも2ヵ月を要することが判ってきた[7)]。上皮化の遅延は創部感染を惹起する危険性があり，次のステップとして解決すべき課題は，上皮化を加速する方法の開発であり，我々は気管上皮細胞や線維芽細胞と人工材料からなるハイブリッド型のスキャフォールド材料の開発に力を注いでいる。さらには形態の複雑な喉頭，特に声帯の再生も重要課題であり，喉頭内腔を型どりした材料を作製して基礎実験を進めている。また，現在はまだ部分切除例のみにしか臨床応用されていないが，今後は切除範囲がより大きな例

にも本再生治療を適応とし，手術手技を確立する必要がある。これらの問題点を克服することで，さまざまな気道欠損に対してより効果的な再生治療を実現できると考えられる。

まとめ

甲状腺癌の気道浸潤例に対する即時再建において，スキャフォールドの移植による組織工学的手法を用いた「気道の再生医療」について紹介した。筆者らは体内で自己組織の再生を誘導する *in situ* tissue engineeringの考え方で行っている。スキャフォールド材料を開発し動物実験で良好な気道の組織再生が得られた成果をもとにして，2002年，筆者らは「気道の再生医療」を世界に先駆けて行い，現時点では良好な結果を得ている。解決すべき次の重要課題としては，上皮化を加速，喉頭の内腔形態の再現，より大きな切除範囲への対応などがあげられる。

癌の治癒切除後のQOLの向上は国民の健康福祉の大きな目標である。組織工学的手法による「気道の再生医療」を癌治療後の機能的再建に応用することで，より効果的でより安全性の高い革新的な治療法を提供することが可能となるであろう。

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総　説

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「第106回日本耳鼻咽喉科学会総会シンポジウム」

耳鼻咽喉科疾患治療の最前線
頭頸部領域の再生医療

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既存の医療の限界から再生医療に対する期待が高まっている．再生医療は，疾患，外傷，先天奇形など不可逆的な損傷を受けた組織・器官を細胞を用いて治療する医療である．再生医療の元になる再生医学の基盤は，医学と工学とが融合して生まれた組織工学にある．組織の再生には細胞，足場，調節因子の3要素が適切な生体環境の下に置かれることによって可能となるといわれている．したがって，いかにこれらの要素を組み合わせてゆくかが組織再生の鍵となるが，これを工学的手法で可能にするのが組織工学である．

組織工学における組織再生のアプローチのひとつとして，上記の再生の3要素を生体内に直接移植するという *in situ* tissue engineering の概念が登場した．この概念によれば，特定臓器の局所再生にあっては生体から供給される以外の要素のみの投与でも再生が可能である．

華々しい再生医学研究の進歩にもかかわらず，現在，再生医療として臨床応用されているものはまだ少ない．*in situ* tissue engineering の概念によって臨床応用へのハードルがやや下がったとはいえ，頭頸部領域での臨床応用ではとくに少ないのが現状である．

本稿では，頭頸部領域の再生研究と臨床応用の現状とについて紹介する．

キーワード： 再生医療，細胞療法，組織工学，生体内組織工学

はじめに

再生医学の登場は，現代医学・医療を根本から変える可能性を示し，それに伴い再生医療に対する社会の関心も高まっている．とくにわが国のように急速に高齢化が進んでいる社会では，既存の医療の限界から未来型の医療とでも言うべき再生医療に対する期待は非常に高い．

一般に高齢化が進むにつれて，人口に占める有病者の割合（有病率）が高まる．臓器の機能障害が軽度なレヴェルにあるときには，投薬や既存手術により機能回復が可能となるが，不可逆的損傷にまで陥ると臓器移植ないしは人工臓器による代替が必要となる．しかし，臓器移植はドナーの問題から絶対的供給量の不足と移植に伴う拒絶反応など大きな障壁あることから，現実的にはほとんど機能していない．また，人工臓器については，人工内耳，ペースメーカーや人工透析など特定の領域を除きその応用範囲に限界がある．これらを背景に，細胞なかでもさまざまな種類の細胞に分化できる能力を有する幹細胞を利用して，機能不全に陥った臓器を再構築するという再生医学に注目が集まった．

再生医学の概念の源は，1950年代に欧米を中心に行われた人工臓器開発のための研究にある．人工臓器を体内に移植するという観点から組織親和性の高い素材の研究開発が行われ，工学と融合した組織工学という新しい医学の分野が生まれるに至たり，これが現在の再生医療の基盤をなしている．また，再生医療の発想そのものは非常にシンプルなもので，拒絶反応のない自己の細胞を用いて人工的に作り出した臓器を部品として，機能不全に陥った臓器の修復を行うというものである．

本稿では，再生医療を支える組織工学の概念と頭頸部領域への臨床応用の現状を取り上げて概説する．

Ⅰ．組織工学

再生医療の基盤ともいえる組織工学（Tissue engineering）[1)2)3)]は，（組織）再生医工学とも呼ばれる．組織・臓器を再生させるためには，図1に示したように，組織再生のもとになる細胞とともに，それが分化・成長

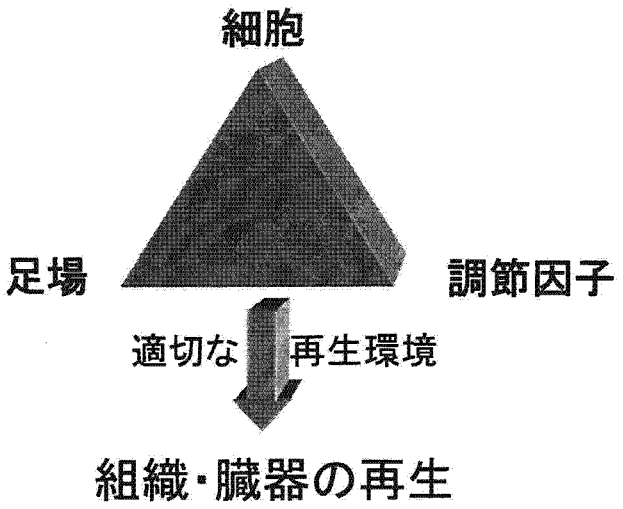

図1　組織工学の3要素
組織再生に要する3要素で，組織再生の元になる細胞（幹細胞），細胞が成長する足場，足場における細胞の成長を調節する因子の3つが，豊富な血流などの良好な再生環境におかれたときに組織再生が可能であるというもの．

するための足場と，それらを調節する因子を適切な環境におくことが必要となる．この細胞，足場，調節因子の3つの要素を組織工学の3要素と呼んでいるが，これらをいかに用いるかが再生の要となる．この3要素についての詳細は，参考文献を参照していただきたい[4]．

組織再生のアプローチには，主として2種類の方法がある．すなわち上記の3要素を用いて，あらかじめ体外（培養器）で組織再生を進めた後，体内に移植する方法と，はじめから再生を目的とする場に3要素を移植して生体内で組織再生を図る方法とである．

前者はハーバード大学のJ. VacantiとMITのR. Langerらが提唱した組織工学のオリジナルの考え方で，この概念が確立されたのは1980年代の後半から90年にかけてである[1)2)3)]．彼らは組織工学の概念を分かりやすく示すために，ヒトの外耳がネズミの背中に生えている衝撃的な写真を論文に掲載した．すなわち，体外（培養器内）で作製した人工的な外耳をネズミの背中に移植して見せたのである．これは世界中の研究者の注目を集め，今日の再生医学研究の隆盛をもたらした．一方後者は，近年わが国を中心に発展してきた概念で，*in situ* tissue engineeringと呼ばれるものである[5)〜8)]．この考えの根幹を成すのは，再生すべき組織を体外で創るのではなく，必要な材料を供給することによって生体内で再生を図ろうとするものである．再生を目的とする組織・臓器にもよるが，生体内では再生に必要な要素のうち自ずと供給される要素が想定される場合には，必ずしもすべての要素が必要なわけではない．すなわち再生の場から供給されるもの以外の要素を移植（供給）すればよいと考えられる．たとえば，神経の再生などでは切除された神経の中枢側から再生神経が伸張してくるために，再生のための細胞の移植は必要ないと思われる．したがって，再生神経線維が伸張できる良好な環境としての足場あるいは，足場と神経成長因子などの投与のみで神経再生が可能であると考えられる．

Ⅱ．臨床応用の現状

再生医学は，近年の組織工学，分子生物学，発生遺伝学などのめざましい進歩によって支えられ発展してきたが，これを臨床に応用する段階となると，たとえば細胞移植の安全性，採取する細胞の出所や拒絶反応など，医学的のみならず倫理的，社会的問題が山積しているため，現在はまだ一部の領域でしか進んでいない．現在実際に再生医療として組織再生法が確立しているのは，皮膚，粘膜，角膜，骨，軟骨など一種類の細胞から構成される単純な構造物としての組織のみである．複数の細胞で構成された機能的な臓器たとえば肝臓や膵臓などの再生はこれまでの技術だけでは不十分である．

耳鼻咽喉科・頭頸部領域では内耳再生などの一部の分野でのみ旺盛な研究が行われているがまだ臨床応用には至っていない．一方，下顎骨[9)10)]，気管[5)7)]，神経[6)11)]など極く限られた領域では，*in situ* tissue engineeringに基づく足場のみあるいは足場と成長因子の移植による臨床応用が開始されている．

今後，現在の手法で再生医療が定着し治療の大きな柱となるか否かは未知であるが，数多くの研究で組織・臓器が再生するということの糸口は見つかっている．おそらくさほど遠くない将来には，再生医療が現行の医療を変える新しい医療のひとつとなると思われる．

Ⅲ．頭頸部領域の再生研究の現状

先にも述べたが頭頸部領域の再生として研究されている対象臓器は非常に限られており，そのほとんどが骨と神経の再生に集中している．しかも，骨の再生は口腔外科や整形外科が神経の再生は脳外科や整形外科が研究を行い，骨再生研究の一環としてそれを顎・顔面骨に応用，あるいは末梢神経再生研究の一環としてこれを顔面神経に応用するなどといったものがほとんどで，世界的にも耳鼻咽喉科・頭頸部外科が主体で研究を行っている施設は，非常に限られている．

現在，頭頸部領域で再生研究が行われているものを表1に示した．このうち臨床応用が開始されているものはわずかに＊印で示した眼窩底[10)]，気管[5)7)]，顔面神経，