GENERAL THORACIC SURGERY

THE EXPERIMENTAL REPLACEMENT OF A CERVICAL ESOPHAGEAL SEGMENT WITH AN ARTIFICIAL PROSTHESIS WITH THE USE OF COLLAGEN MATRIX AND A SILICONE STENT

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Objective: **Attempts have been made to replace esophageal defects with a variety of artificial materials. However, because of the artificial nature of the materials, problems such as infection, leakage, stricture, or dislocation could not be avoided. Therefore we have designed a new type of artificial esophagus that is gradually replaced by host tissue.** ***Methods:*** **Our artificial esophagus was a two-layered tube consisting of a collagen sponge matrix and an inner silicone stent. We used it to replace 5 cm esophageal segmental defects in 43 dogs, and the inner silicone stent was removed endoscopically at weekly intervals from 2 to 4 weeks.** ***Results:*** **In the 27 dogs from which the silicone stent was removed at 2 or 3 weeks, constriction of the regenerated esophagus progressed and the dogs became unable to swallow within 6 months. In the 16 dogs from which the silicone stent was removed at 4 weeks, highly regenerated esophageal tissue successfully replaced the defect, leaving no foreign body in the host. Moreover, the regenerated esophagi had stratified flattened epithelia, striated muscle tissue composed of an inner circular and an outer longitudinal muscle layer, and esophageal glands.** ***Conclusions:*** **Even in mature adult higher mammals, esophageal high-order structures can be regenerated provided that an adequate three-dimensional extracellular structure is put in place for a sufficient period. (J Thorac Cardiovasc Surg 1998;116:98-106)**

Reconstructive surgery for esophageal cancer is still associated with many complications. Various artificial esophagi have been developed in attempts to minimize invasive surgery for patients, but they are associated with complications, such as leakage, infection, stenosis, and dislocation of the artificial esophagus. Therefore these devices have proven to be far from useful in clinical practice.[1]

We have designed a new type of artificial esophagus comprising a silicone tube covered with nonantigenic collagen. The novel feature of this prosthesis is that it does not remain at the implantation site, but is replaced by regenerated host tissue.

Our previous study showed that when a 5 cm segmental defect of the cervical esophagus of an adult mongrel dog was replaced with our artificial esophagus, autologous, continuous, cylinder-shaped, connective tissue regeneration was accomplished after 2 weeks. However, removal of the silicone stent from the esophageal prosthetic lumen resulted in immediate contraction and shortening of the regenerated esophagus. Histologically, the rough submucosal tissue that had appeared changed to tight, hard connective tissue. Consequently, no high-structure regeneration occurred.[2] For luminal constriction and scar formation to be avoided, a three-dimensional space must be maintained to enable the submucosal rough tissue to develop fully. Therefore we decided that the silicone tube should be left in situ for a certain time after the new epithelium had formed. The object of this study was to examine the effect of the duration of silicone stent retention in the prosthetic lumen on submucosal tissue formation.

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Materials and methods

Collagen. Collagen was extracted from pig skin (6 months after birth), purified by enzymatic treatment as described herein, and solubilized in a 10 mmol/L concentration of acetic acid to produce a final concentration of 1% wt/vol. Pig skin, from which the hair and fat had been removed, was chopped into small pieces, homogenized with a Polytron homogenizer (Kinematida, Switzerland), suspended in cold 0.01N hydrochloric acid for 24 hours, and solubilized with pepsin (substrate/enzyme ratio, by weight, 100:1) at 4° C for 48 hours. The resulting dispersion was centrifuged at 7000*g* for 20 minutes and the pellet was discarded. The supernatant consisted predominantly of type I collagen (70% to 80%) and the rest was type III (confirmed by sodium dodecylsulfate–polyacryamide gel electrophoresis [SDS-PAGE]).

Artificial esophagus. A silicone tube, 5 cm long, with an internal diameter of 2.5 cm, was made from a medical-grade, 1 mm thick silicone sheet (Silastic; Dow Corning Corp., Midland, Mich.), reinforced by embedding nylon mesh and fixed with silicone glue (Silastics Medical Adhesive Silicone Type A; Dow Corning).

The outer surface of the tube was exposed to a coronal discharge at 9 kV for 5 minutes to make the surface hydrophilic, and then the silicone tube was placed inside a Teflon tube with an internal diameter of 3 cm. The collagen solution, which had been bubble-stirred at 3000 rpm in a refrigerated homogenizer for 20 minutes, was poured between the Teflon and the silicone tube, freeze-dried at about –20° C to produce a porous sponge, and then heated at 105° C under vacuum for 24 hours to introduce light cross-linking between the collagen molecules, resulting in a microstructure. In general, native collagen sponge is liable to swell when exposed to body fluids and is solubilized easily, and the above process was necessary to ensure that the highly porous structure was maintained during suturing and handling. The collagen was subjected to no further chemical modification so that its good biocompatibility would be retained.

The pore diameters of the collagen sponge were controlled to within the range of 100 to 500 μm (Fig. 1). Finally, the outer Teflon tube was removed and the collagen silicone composite tube was sterilized with ethylene oxide gas.

Operative and postoperative procedures. Forty-three adult mongrel dogs weighing 7 to 12 kg were anesthetized and placed in the supine position. Then a median cervical skin incision was made and the trachea and cervical esophagus were isolated carefully to avoid injuring the bilateral truncal vagus. The esophagus was clamped in two places, a 5 cm long piece was excised, the prosthesis was placed between the distal and proximal stumps, and monolayer end-to-end anastomosis with interrupted 3-0 Prolene sutures (Ethicon, Inc., Somerville, N.J.) was performed. To determine the length of the regenerated esophagus by radiography and also to indicate that no constriction had occurred at the replaced part, we placed surgical metal clips at each end as markers (Fig. 2). After implantation of the prosthesis, an intravenous hyperalimentaion tube was inserted into the left femoral vein and the dogs were fed only by intravenous hyperalimentation with 80 calories per kilogram and 60 ml of water per kilogram per day, using an infusion pump, for 2 to 4 weeks after the operation. Forty-three dogs were separated into three groups. In group A, the silicone tubes were dislodged endoscopically 2 weeks after the operation and oral feeding was started (12 dogs). In group B, the silicone tubes were dislodged endoscopically 3 weeks after the operation and oral feeding was started (15 dogs). In group C, the silicone tubes were dislodged endoscopically 4 weeks after the operation and oral feeding was started (16 dogs). Dogs were put to death at various intervals from 2 weeks to 12 months after prosthesis implantation, and neoesophageal tissue formation was examined.

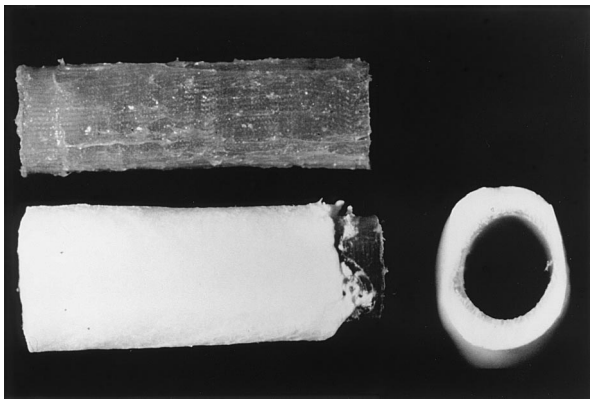

Fig. 1. Bilayered artificial esophagus consisting of collagen sponge and silicone.

The experiment was carried out in accordance with the Animal Experiment Guidelines of Kyoto University, 1989.

Histopathology. Tissue sections were fixed with 10% vol/vol buffered neutral formalin and processed for histologic examination with standard elastic van Giesen and Masson's trichrome staining procedures. The distal anastomoses were examined by means of routine microscopic techniques.

Immunostaining. An antidesmin antibody, raised against purified desmin isolated from bovine Purkinje fibers, was obtained from Labsystems Inc. (Rochester, N.Y.) and used for immunostaining by the peroxidase antiperoxidase (PAP) method. After treatment with 3% vol/vol hydrogen peroxide in methanol for 25 minutes and trypsinization with a mixture of 1% wt/vol trypsin and 1% wt/vol calcium chloride for 30 minutes at room temperature, the sections were incubated at room temperature with the monoclonal mouse immunoglobulin G antibody against desmin, diluted 1:175 with phosphate-buffered saline solution. The sections were incubated for 45 minutes at room temperature with peroxidase-conjugated rabbit anti-mouse immunoglobulin (Dakopatts; P260), diluted 1:50 with phosphate-buffered saline solution containing 1% vol/vol bovine serum albumin. The sections were stained with diaminobenzidine and counterstained with hematoxylin. All the above incubations were carried out with gentle agitation in a humidity chamber.

Transmission electron microscopy. Fixed explants were removed from osmium tetroxide in a 0.1 mol/L concentration of phosphate buffer, pH 7.4, embedded in

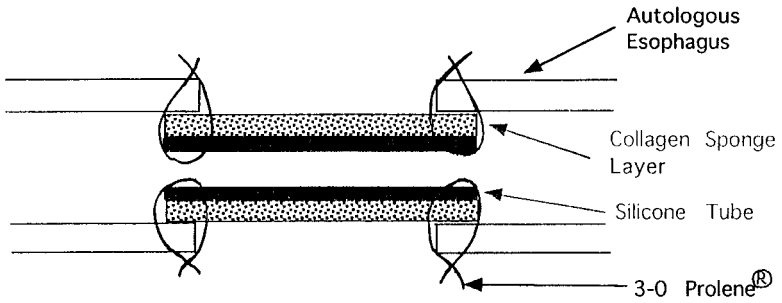

Fig. 2. The operative procedures. A 5 cm long piece of esophagus was excised between the clamps and the artificial esophagus was anastomosed to the distal and proximal stumps.

Table I. *Result of group A*

Animal No.	*Survival time (days)*	*Duration of stent (days)*	*Prognosis and death*	*Regeneration*		
				Epithelium	*Muscle*	*Glands*
1	14	14	Death	Partial	−	−
2	16	14	Death	Partial	−	−
3	15	14	Death	Partial	−	−
4	17	14	Death	partial	−	−
5	16	14	Death	Partial	−	−
6	15	14	Death	Partial	−	−
7	16	14	Death	Partial	−	−
8	14	14	Death	Partial	−	−
9	16	14	Death	Partial	−	−
10	15	14	Death	Partial	−	−
11	17	14	Death	Partial	−	−
12	15	14	Death	Partial	−	−

Minus sign (−), not present.

Epon fixative, and serial sections (3 μm thick) were cut and stained with toluidine blue. The thin sections were studied using a Japan Electronics Optics Limited JEM-1200 EX.

Result

The stents of the 12 dogs in group A were removed 2 weeks after the operation and oral feeding was started. Within 3 days of stent removal, constriction of the regenerated esophagus progressed along the longitudinal and horizontal axes, and oral feeding became impossible (Table I). Epithelial regeneration started from both esophageal stumps, but the centers of the regenerated esophagi had not become reepithelialized. The submucosal layer of each regenerated esophagus was occupied by scar tissue and hard connective tissue, but neither esophageal glands nor a muscle layer was present (Fig. 3, *A*). Once the submucosal tissue had changed to scar tissue and then become granular tissue, no high-order structures appeared.

The stents of the 15 dogs in group B were removed 21 days after the operation and oral feeding was started. For the histologic evaluation, five dogs were put to death at this point. The regenerated esophagi were completely covered with layers of stratified flattened epithelium, the epithelial regeneration originating from both esophageal stumps. The stratified flattened epithelium had regenerated from the oral and distal ends, comprised six or eight layers, and beneath it, regenerated esophageal glands were present (Fig. 3, *B*). When the stent was removed, almost all the submucosal tissue was found to be rough connective tissue, and immature muscle tissue had regenerated close to the regenerated esophagus on both sides. In other dogs, constriction of the regenerated esophagus progressed gradually along the longitudinal and horizontal axes after stent removal, and the dogs became unable to swallow within 6 months (Fig. 3, *C*). No muscle tissue was present in the submucosal tissue

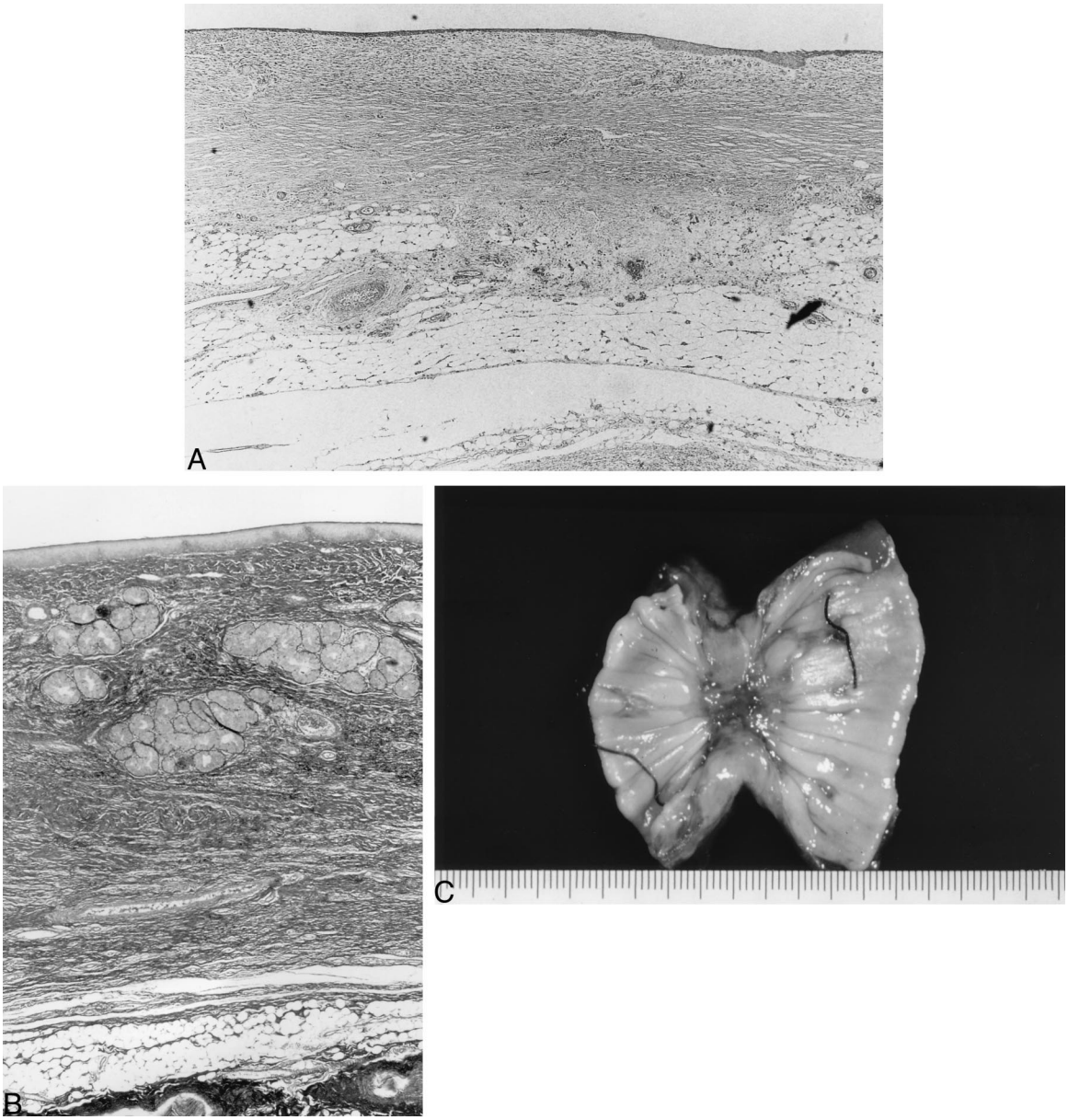

Fig. 3. A, Regenerated esophagus 14 days after artificial esophagus implantation. Note the connective tissue. (Masson's trichrome stain, original magnification ×200.) **B,** The stent was removed 21 days after the operation, oral feeding was resumed, and the dog was put to death 3 days later. Note the regenerated esophageal glands. (Elastic van Gieson stain, original magnification ×100.) **C,** The stent was removed 21 days after the operation, oral feeding was resumed, and the dog was put to death 6 months later. Note the internal surface of the neoesophageal lumen.

at the stenotic site. Consequently, although complete reepithelialization occurred, scar formation was not prevented by leaving the stent in situ for 3 weeks (Table II).

The stents of group C dogs were removed 4 weeks after the operation. Six of the 16 dogs of group C were put to death when the stent was removed; for the remaining 10 dogs oral feeding was started. They survived and oral feeding was possible. The regenerated esophagus was completely covered with new stratified flattened epithelium comprising eight to ten layers, beneath which both mature and imma-

Table II. *Result of group B*

Animal No.	*Survival time (days)*	*Duration of stent (days)*	*Prognosis and death*	*Regeneration*			*Stenosis*
				Epithelium	*Muscle*	*Glands*	
1	21	21	Put to death	Complete	−	+	−
2	21	21	Put to death	Complete	−	+	−
3	21	21	Put to death	Complete	−	+	−
4	21	21	Put to death	Complete	−	+	−
5	21	21	Put to death	Complete	−	+	−
6	43	21	Death	Complete	−	+	+
7	33	21	Death	Complete	−	+	+
8	65	21	Death	Complete	−	+	+
9	90	21	Put to death	Complete	−	+	+
10	54	21	Death	Complete	−	+	+
11	34	21	Death	Complete	−	+	+
12	90	21	Put to death	Complete	−	+	+
13	86	21	Death	Complete	−	+	+
14	172	21	Death	Complete	−	+	+
15	140	21	Death	Complete	−	+	+

Plus sign (+), present; *minus sign* (−), not present.

Table III. *Result of group C*

Animal No.	*Survival time (mo)*	*Duration of stent (days)*	*Prognosis and death*	*Regeneration*			*Stenosis*
				Epithelium	*Muscle*	*Glands*	
1	1	28	Put to death	Complete	+	+	−
2	1	28	Put to death	Complete	+	+	−
3	1	28	Put to death	Complete	+	+	−
4	1	28	Put to death	Complete	+	+	−
5	1	28	Put to death	Complete	+	+	−
6	1	28	Put to death	Complete	+	+	−
7	3	28	Put to death	Complete	+	+	−
8	3	28	Put to death	Complete	+	+	−
9	6	28	Put to death	Complete	+	+	−
10	6	28	Put to death	Complete	+	+	−
11	12	28	Put to death	Complete	+	+	−
12	12	28	Put to death	Complete	+	+	−
13	12	28	Put to death	Complete	+	+	−
14	12	28	Put to death	Complete	+	+	−
15	12	28	Put to death	Complete	+	+	−
16	12	28	Put to death	Complete	+	+	−

Plus sign (+), present; *minus sign* (−), not present.

ture esophageal glands arose from regenerated epithelium. In the lower submucosal tissue, the regenerated striated muscle tissue was composed of two layers, an inner circular layer and an outer longitudinal muscle layer (Fig. 4, *A*). All the regenerated muscle tissue was striated muscle, confirmed by immunostaining with antidesmin and mouse anti-rabbit stains.

Alpha-sarcomeric muscle actin antibodies[3] (Fig. 4, *B*). In group C, stenosis did not occur in any dogs in which the stents remained in place for 4 weeks, and subsequent oral feeding remained possible, without weight loss, even 12 months after the operation (Table III). Radiographic examination showed no stenosis or shortening at the replaced part (Fig. 5, *A*). The stratified flattened epithelia were six or eight layers thick 3 months later and were indistinguishable from the native esophageal epithelia. Mature esophageal glands and muscle tissue had regenerated in the submucosal tissue (Fig. 5, *B*). Electron microscopy revealed that the muscle fiber thickness and density of the normal and regenerated esophagi were the same (Fig. 5, *C*). By 3 months after the operation, the entire length and all the layers of the 5 cm esophageal segmental defect had been replaced by regenerated tissue with a structure iden-

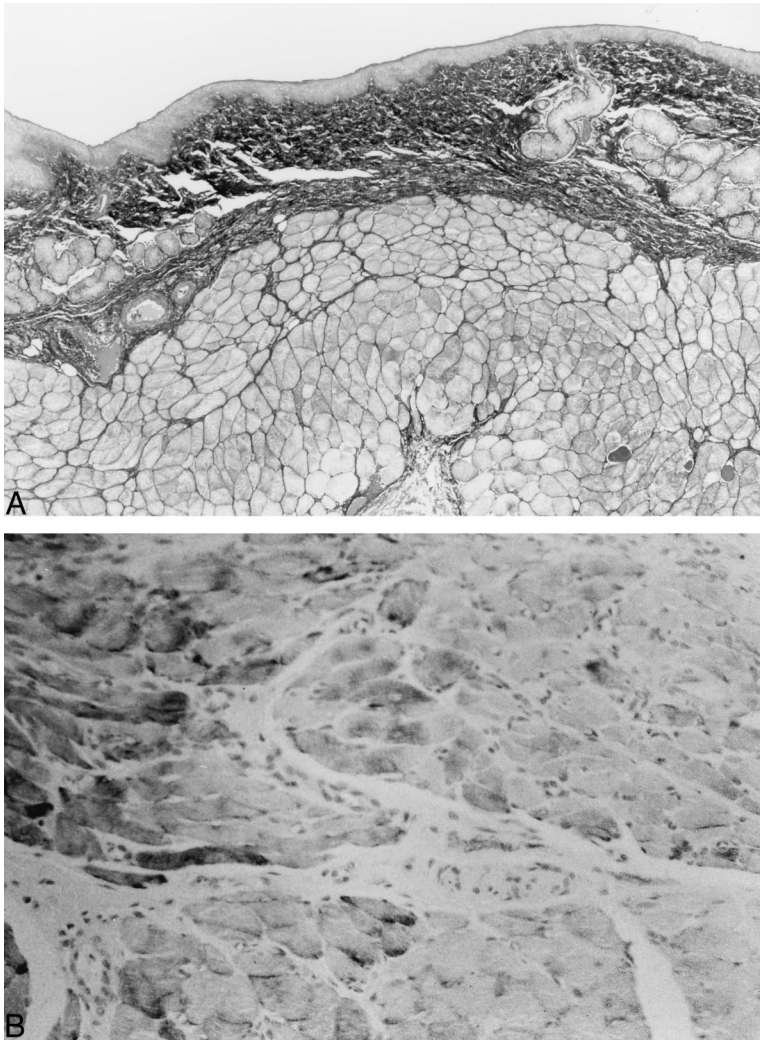

Fig. 4. A, Regenerated esophagus 28 days after artificial esophagus implantation. Note the regenerated muscle tissue and inner circular and outer longitudinal muscle layers. (Masson's trichrome stain, original magnification ×100.) **B,** Regenerated esophagus 28 days after artificial esophagus implantation. The regenerated muscle tissue was immunostained by the antidesmin antibody, which specifically stains striated muscle fibers. (Original magnification ×400.)

tical to that of the native esophagus. Six and 12 months after the operation, the regenerated esophagus had not changed (Fig. 5, *D*).

Discussion

The goal of this investigation was to obtain regenerated high-order tissue structures that replace the host tissue defect. However, in adult higher animals, such as mammals, damaged structures generally do not regenerate, whereas damaged legs of adult amphibians, such as newts, can regenerate from the blastema formed in the stump. Without this blastema, regeneration of high-order structures of the legs, such as muscle cartilage and bone, does not occur. The blastema is composed of an extracellular matrix, which provides a scaffold on which cells can proliferate and maintains certain conditions essential for the regeneration of high-order structures.[4-9] Previous studies have shown that damaged organs with simple tissue structures of higher mammals can also regenerate in vivo as a result of cell proliferation.[10-13] However, to the best of our knowledge, the regeneration of complex tissue structures, such as muscles, nerves, blood vessels, and epithelia, has

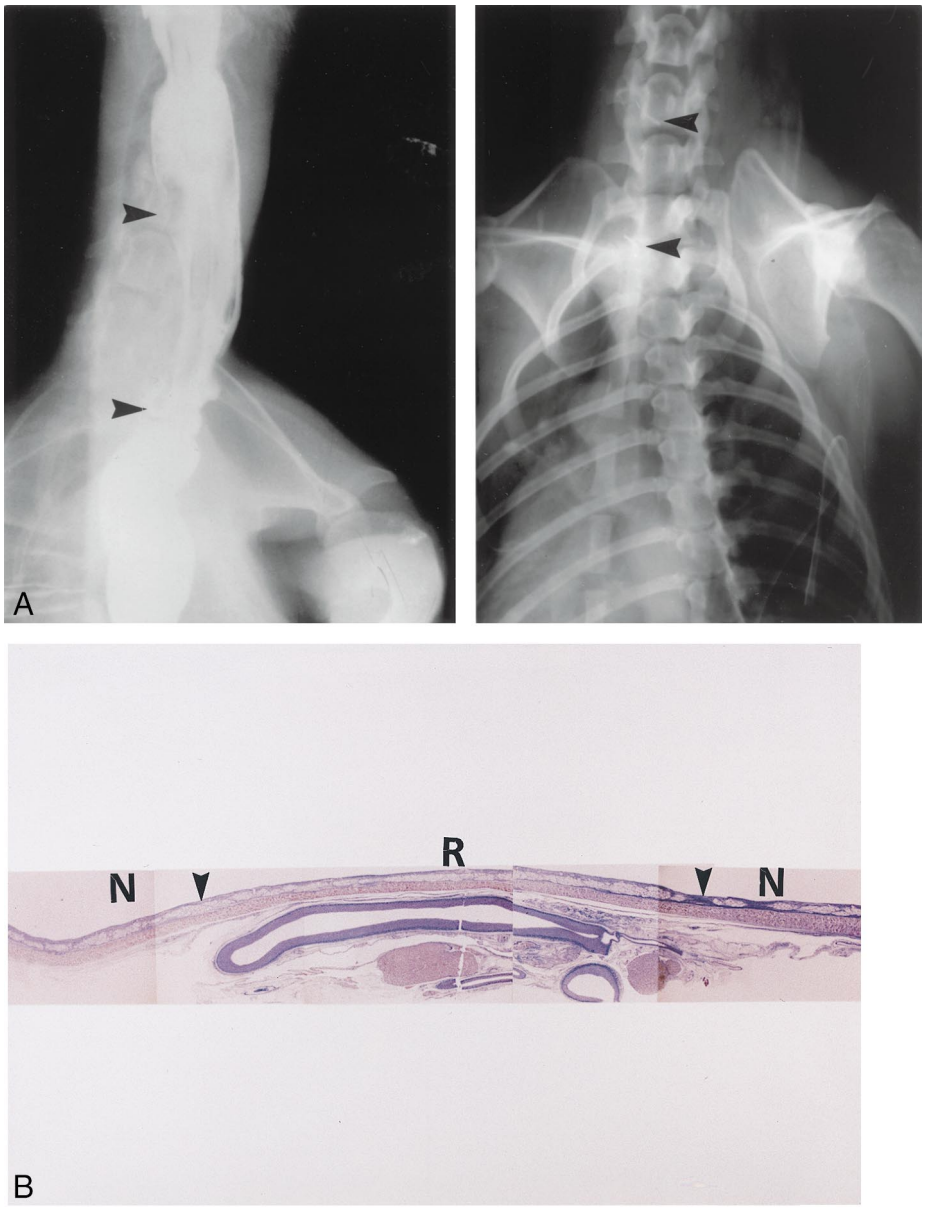

Fig. 5. A, Radiographs taken 12 months after implantation, showing no stenosis or shortening of the neoesophagus. Two *arrowheads* show the interposed area. **B,** Regenerated esophagus 3 months after artificial esophagus implantation. Note the regenerated esophagus *(R)* and the native esophagus *(N).* Two *arrowheads* show the distal and proximal stumps. (Masson's trichrome stain, original magnification ×1.)

never been demonstrated in vivo. When an organ is damaged, the wound undergoes scar formation and is subject to contraction forces. Consequently, high-order structures like those described herein do not regenerate.[14, 15] In this study we provided an extracellular matrix, which promotes epithelial and connective tissue regeneration and is essential for tissue generation, by coating the prosthesis with collagen. When 5 cm segmental cervical esophageal defects in adult mongrel dogs were replaced with an artificial esophagus, autologous epithelial regeneration at the sites of both stomas occurred and the defect was replaced completely by a cylinder of regenerated tissue within about 3 weeks. However, when the silicone stent that kept the inner space open was removed after completion of reepithelialization, the

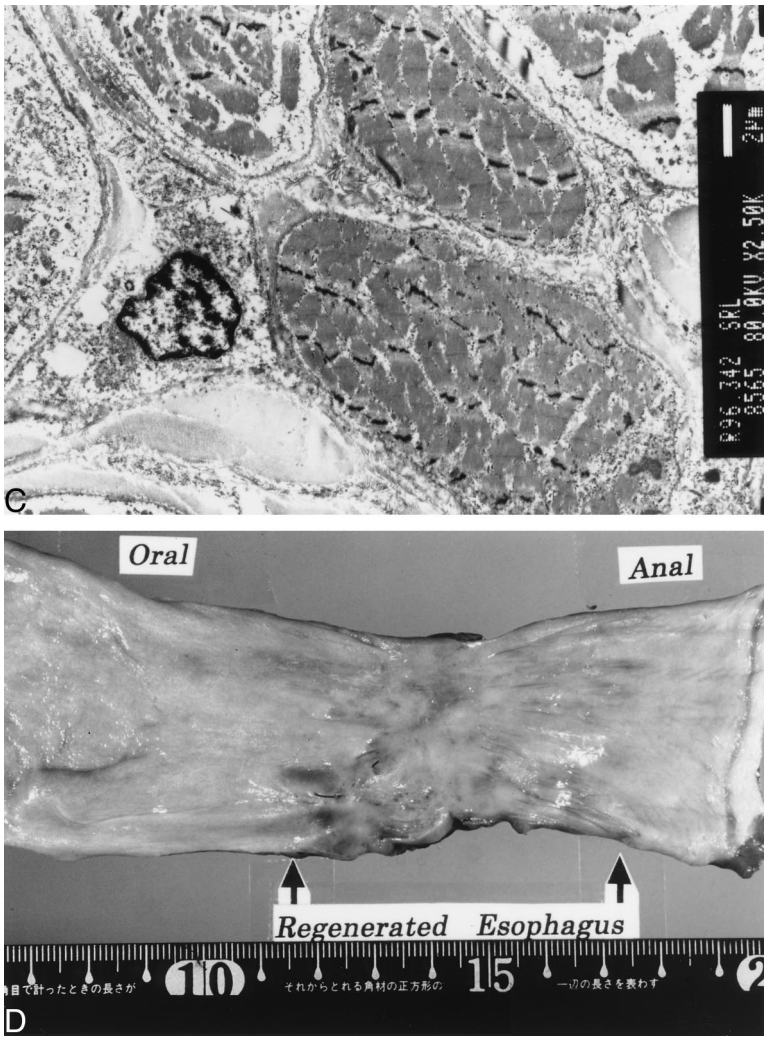

Fig. 5. Cont'd. C, Regenerated esophagus 3 months after the operation. The mature striated muscle tissue has regenerated. **D,** Regenerated esophagus 12 months after artificial esophagus implantation. The regenerated esophagus is indistinguishable from the native esophagus. Two *arrows* show the distal and proximal stumps.

regenerated esophagus contracted and immediately shortened. We believe that the stenosis occurred as a result of poor-quality submucosal tissue. Therefore we controlled the scar formation mechanism by leaving a silicone stent in the prosthetic lumen for a longer time after the operation. When we replaced esophageal segmental defects in dogs, as representative adult higher animals, with our artificial esophagi and removed the inner silicone tubes 28 days after the operation, well-developed esophagi had regenerated including esophageal mucosa, glands, and striated muscle, and without foreign body remaining. Under such conditions, high-order structure can regenerate from an esophageal segmental defect in an adult mammal.

It was necessary to distinguish whether the muscle that appeared was due to regeneration or to constriction by the scar tissue. At the anastomotic end, whole layers of tissue were fixed on a silicone tube with sutures at 5 mm intervals. Therefore, during stenting, the bilateral ends of the autologous esophagus could not have been constricted by connective tissue. In addition, to rule out constriction of the replaced segment, we marked both ends of the resected esophagus with a metal clip. That is, at the anastomotic end, all the layers in the free region

between the suture points were grasped by a metal clip. We took radiographs during the operation, on removal of the stent, and after removal, and confirmed that longitudinal constriction had not occurred at the replaced part. These facts suggest that the replaced area was not the constricted tissue.

The mechanism involved in tissue repair by promoting self-regeneration of absent host tissue is often accompanied by scar tissue contraction in an attempt to hasten repair. If this happens, high-order structures do not regenerate. The body consists of cells and extracellular matrices that support cells. The extracellular matrix plays an important role in tissue regeneration. This study also suggests that self-regeneration of tissue defects can be promoted with collagen matrix and that high-order structures can regenerate if an appropriate environment is provided. In this self-regeneration process, a three-dimensional extracellular matrix structure should be maintained for an adequate length of time.

REFERENCES

1. Barnes WA, Redo SE, Ogata K. Replacement of portion of canine esophagus with composite prosthesis and greater omentum. J Thorac Cardiovasc Surg 1972;64:892-6.
2. Natsume T, Ike O, Okada T, Takimoto Y, Shimizu Y, Ikada Y. Porous collagen sponge for esophageal replacement. J Biomed Mater Res 1993;27:867-75.
3. Leader M, Collins M, Patel J, Henr K. Desmin: its value as a marker of muscle derived tumors using a commercial antibody. Virchows Arch A 1987;411:345-9.
4. Bryant SV. Regenerative failure of double half limbs in *Notophthalamus viridescens.* Nature 1976;263:676-9.
5. Dollé P, Izpisòa-Belmante JC, Falkenstein H, Remucci A, Duboule D. Coordinate expression of the murine Hox-5 complex homobox-containing genes during limb pattern formation. Nature 1989;342:767-72.
6. Yokouchi Y, Sasaki H, Kuroiwa A. Homeobox gene expression correlated with bifurcation process of limb cartilage development. Nature 1991;353:443-5.
7. Tickle C, Summerbell D, Wolpert L. Positional signaling and specification of digits in chick limb morphogenesis. Nature 1975;254:199-202.
8. Niswander L, Jefrey S, Martin GR, Tickle C. Positive feedback loop coordinates growth and patterning in the vertebrate limb. Nature 1994;371:609-12.
9. Parr BA, McMahon AP. Dorsaling signal Wnt-7a required for normal polarity of D-V and A-P axes of mouse limb. Nature 1995;374:350-3.
10. Geislaler A, Stiller K, Machnik G. The cellular reproduction in physiological and reparative lever regeneration. Exp Toxicol Pathol 1994;46:247-50.
11. Rhim JA, Sandgren EP, Degan JL, Palmiter RD, Brinster RL. Replacement of diseased mouse liver by hepatic cell transplantation. Science 1994;263:1149-52.
12. Bell E, Ehrlich HP, Buttle D, Nakatsuji T. Living tissue formed in vitro and accepted as skin equivalent tissue of full thickness. Science 1981;21l:1052-4.
13. Hasegawa T, Hirose T, Kudo E, Abe J, Hizawa K. Cytoskeletal characteristics of myofibroblasts in benign neoplastic and reactive fibroblastic lesions. Virchows Arch A Pathol Anat Histopathol 1990;416:375-82.
14. Stratmann U, Schaarschmidt K, Lehmann RR, Willital GH, Wessling G, Kessler T. The cell response of rat esophagus after intraluminal irradiation with Nd-YAG-laser (1064nm) after various postoperative times: a light and electron microscopic study. Anat Anz 1993;175:95-100.
15. Carlson BM, Shepard B, Komorowski TE. A histological study of local anesthetic-induced muscle degeneration and regeneration in the monkey. J Orthop Res 1990;8:485-94.