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Clinical Papers

Patch esophagoplasty using an in-body-tissue-engineered collagenous connective tissue membrane

Hiroomi Okuyama [a,*], Satoshi Umeda [a], Yuichi Takama [a], Takeshi Terasawa [b], Yasuhide Nakayama [b]

[a] Department of Pediatric Surgery, Osaka University Graduate School of Medicine, Osaka, Japan
[b] Division of Medical Engineering and Materials, National Cerebral and Cardiovascular Centre Research Institute, Osaka, Japan

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ABSTRACT

Aim: Although many approaches to esophageal replacement have been investigated, these efforts have thus far only met limited success. In-body-tissue-engineered connective tissue tubes have been reported to be effective as vascular replacement grafts. The aim of this study was to investigate the usefulness of an In-body-tissue-engineered collagenous connective tissue membrane, "Biosheet", as a novel esophageal scaffold in a beagle model.

Methods: We prepared Biosheets by embedding specially designed molds into subcutaneous pouches in beagles. After 1–2 months, the molds, which were filled with ingrown connective tissues, were harvested. Rectangular-shaped Biosheets (10×20 mm) were then implanted to replace defects of the same size that had been created in the cervical esophagus of the beagle. An endoscopic evaluation was performed at 4 and 12 weeks after implantation. The esophagus was harvested and subjected to a histological evaluation at 4 ($n = 2$) and 12 weeks ($n = 2$) after implantation. The animal study protocols were approved by the National Cerebral and Cardiovascular Centre Research Institute Committee (No. 16048).

Results: The Biosheets showed sufficient strength and flexibility to replace the esophagus defect. All animals survived with full oral feeding during the study period. No anastomotic leakage was observed. An endoscopic study at 4 and 12 weeks after implantation revealed that the anastomotic sites and the internal surface of the Biosheets were smooth, without stenosis. A histological analysis at 4 weeks after implantation demonstrated that stratified squamous epithelium was regenerated on the internal surface of the Biosheets. A histological analysis at 12 weeks after implantation showed the regeneration of muscle tissue in the implanted Biosheets.

Conclusion: The long-term results of patch esophagoplasty using Biosheets showed regeneration of stratified squamous epithelium and muscular tissues in the implanted sheets. These results suggest that Biosheets may be useful as a novel esophageal scaffold.

Surgical reconstruction of long-gap esophageal atresia is often associated with a high incidence of morbidity and complications [1]. Although endoscopic therapies are the first choice of treatment and are successful in most cases, refractory strictures are not rare and often need surgical correction. Although many approaches to esophageal replacement using stomach, intestine, and colon have been reported, it has been associated with a high incidence of long-term morbidity in children [2,3].

The alternative for esophageal reconstruction involves patch esophagoplasty. Several kind of degradable extracellular matrix (ECM) scaffolds have recently shown promising results in both preclinical and clinical settings [4–7]. However, patch esophagoplasty using xenogenic ECM in children has not been reported, and its long-term outcomes remain unclear.

In-body tissue architecture (IBTA) advocates the use of in vivo tissue engineering technology to develop autologous implantation tissue. Nakayama et al. reported the successful implantation of small-diameter vascular grafts, called 'biotubes', produced by IBTA technology [8,9]. IBTA technology has been also applied for patch tracheoplasty in a rabbit model, demonstrating that cartilage could self-regenerate onto an airway patch using a Biosheet [10]. However, an autologous patch developed with IBTA has not been used in the esophagus.

The aim of the current study was to examine the remodeling events that occur when an autologous patch developed with IBTA, called a 'Biosheet', is used as a novel esophageal scaffold in a beagle model.

1. Materials and methods

The experimental protocol for patch esophagoplasty using a Biosheet is shown in Fig. 1. All surgical operations were performed under general anesthesia. Preanesthetic medication included ketamine 5 mg/kg intramuscularly (IM), buprenorphine 0.02 mg/kg IM, and atropine sulfate 0.025 mg/kg IM, and anesthesia was induced by pentobarbital 15 mg/kg intravenously (IV) and maintained by bolus induction of pentobarbital IV at a quarter or half of the initial doses. The animal

* Corresponding author at: Osaka University Graduate School of Medicine, Department of Pediatric Surgery, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan. Tel.: +81 6 6879 3753.
E-mail address: okuyama@pedsurg.med.osaka-u.ac.jp (H. Okuyama).

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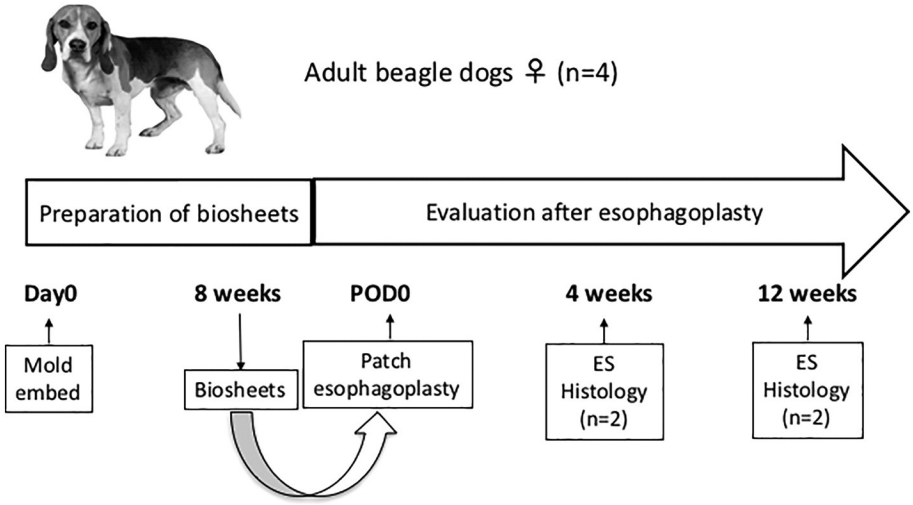

Fig. 1. Experimental protocol for patch esophagoplasty using Biosheets.

study protocols were approved by the National Cerebral and Cardiovascular Centre Research Institute Committee (No. 16048).

1.1. Preparation and the histological examination of the Biosheets

We prepared Biosheets by embedding specially designed molds into subcutaneous pouches in beagles. After eight weeks, the molds, which were filled with ingrown connective tissues, were harvested to obtain the biotubes. After harvesting the molds, the biotubes formed on the silicone rods were obtained by removing the molds and trimming the surrounding peripheral tissues. Rectangular-shaped Biosheets (10 × 20 mm) were obtained by trimming the biotubes (Fig. 2).

Biosheet specimens were fixed in a 10% formalin solution and then embedded in paraffin. The embedded tissues were cut into 3- to 5-μm-thick sections and stained with routine hematoxylin and eosin (H&E) and Masson's trichrome stain for collagen, which showed that the obtained Biosheets were formed as collagen-based tissue (Fig. 3).

1.2. Surgical procedure (patch esophagoplasty)

Each dog was anesthetized by induction with thiopental sodium IV, and surgical plane anesthesia was maintained with 4% isoflurane via an endotracheal tube. A ventral midline incision was made, and the esophagus was exposed via blunt dissection. Partial circumferential resection of the esophagus was performed. The removed section of the esophagus measured 10 mm in width and 20 mm in length for the patch defects. Rectangular-shaped Biosheets (10 × 20 mm) were implanted with interrupted stiches to replace the defects of the same size (Fig. 4). No muscle or skin flaps were utilized. The soft tissue structures of the neck were then closed anatomically.

Each animal received intraoperative antibiotics (cefazolin, 500 mg IV). The animals were not given any food by mouth for a few hours after surgery but were allowed to eat dog food on day 2 after surgery.

1.3. Endoscopic evaluations

Endoscopic evaluations were performed under general anesthesia at 4 and 12 weeks after implantation to evaluate the internal surface of the esophagus and the implanted Biosheets.

1.4. Specimen collection and histological examinations

The esophagus was harvested and subjected to a histological evaluation at 4 (n = 2) and 12 weeks (n = 2) after implantation. Each animal was killed by an overdose of pentobarbital administered IV followed by potassium chloride. The graft site, along with the adjacent normal tissue, was harvested and divided. One section was placed in 10% neutral buffered formalin for a histological examination. The formalin-fixed

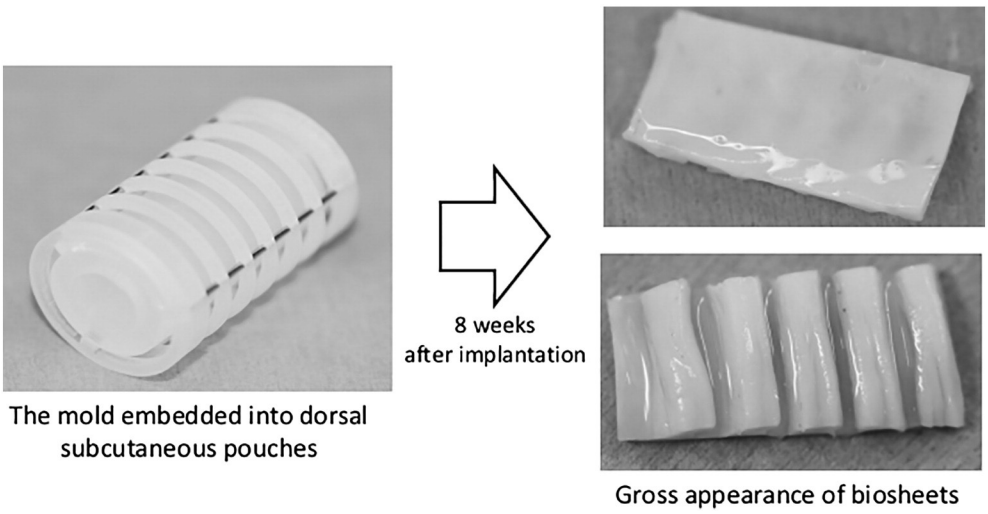

Fig. 2. Preparation of the Biosheets.