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Epidermal Cell Sheet Transplantation on an Anastomotic Site of the Small Intestine in an Experimental Animal Model

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Objective: The present study was performed to examine the effects of anastomotic site tissue reconstruction by transplantation of epidermal cell sheets onto the small intestine in an animal model. Cell sheet engineering using cell sheets are used to construct monolayers and bilayers, which are then transplanted into organs. Clinical trials of the application of cell sheets to the cornea, esophagus, lung, and heart muscle are currently underway.

Methods: The small intestine in female pig (20 kg) was cut 1.5 cm vertically at 6 points at 10-cm intervals, and Gambee sutures were applied at 5-mm intervals. The suture line was covered by epidermal cell sheets. Resection was performed 1 week after the operation.

Results: Cell sheets applied to sutures in the small intestine survived and differentiated 1 week after transplantation. The small intestine showed marked thickening in the region of cell sheet transplantation, and the amount of connective tissue in the transplanted specimens was 2.54 times that in controls.

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Conclusions: Further studies are necessary to identify the strength of anastomosis and substances that may enhance collagen synthesis and healing at sites of anastomosis.

Key words: Tissue reconstruction – Anastomotic leakage – Cell sheet engineering

There have been a number of recent reports indicating clinical success in reconstruction of a wide range of functional tissues using novel biomaterials combined with living cells.[1–3] Okano *et al* reported cell sheet engineering using cell sheets to construct monolayers and bilayers, which are then transplanted into organs[1–3] Poly(*N*-isopropylacrylamide) (PIPAAm) was grafted with poly(ethylene glycol) (PEG) onto porous culture membranes by electron beam eradiation to accelerate the necessary culture substrate hydrophilic/hydrophobic functional changes according to hydrated/dehydrated structural changes in response to alterations in temperature. The complete detachment of cell sheets from PIPAAm-PM under static conditions requires incubation at 20°C for approximately 35 minutes. Immunoblotting analyses of harvested keratinocyte sheets indicated that dispase treatment disrupted E-cadherin and laminin 5, whereas these molecules remained intact in keratinocyte sheets harvested from the grafted dishes under conditions of reduced temperature. Clinical trials of the application of cell sheets to the cornea, esophagus, lung, and heart muscle are currently underway.[4] They will likely be able to remain within the wound and produce appropriate signals and substances for sufficiently long periods to allow restoration of resident cells to achieve proper wound healing.[5–7]

Anastomotic leakage is a severe complication of gastrointestinal surgery for gastrointestinal cancer, which results in prolonged hospitalization period and reduced quality of life after the operation. The results of a nationwide questionnaire survey of anastomotic leakage after rectal cancer conducted by the Japan Colorectal Surgical Club (JCSC) indicated an especially high occurrence rate of leakage associated with surgery for rectal cancer, with a median of 8.0% (range, 0%–27%). Risk factors for anastomotic leakage can be categorized as patient specific, intraoperative, and specific for low rectal anastomosis.[8–11] Patient-specific risk factors include malnutrition, steroid use, smoking, leukocytosis, cardiovascular disease, alcohol use, American Society of Anesthesiologists (ASA) score, and diverticulitis. Intraoperative risk factors include low colorectal anastomosis, suboptimal anastomotic blood supply, operative time >2 hours, bowel obstruction, perioperative blood transfusion, and intraoperative septic conditions not conductive to primary anastomosis. Risk factors for rectal anastomosis include male sex and obesity.

Anastomotic leakage is detected empirically at about 7 days postoperatively, and once recognized, its management should be individualized to accommodate the needs of the patient. A number of strategies are available for anastomotic leakage, including observation and bowel rest, percutaneous drainage, colonic stenting, surgical revision, diversion, and drainage. We previously implemented temporary diversion with intestinal or colonic stoma to prevent anastomotic leakage in cases of low rectal cancer. None of the currently available methods for preventing anastomotic leakage have been widely adopted, and new techniques to strengthen the sites of anastomosis are required (Fig. 1).

The present study was performed to examine the effects of anastomotic site tissue reconstruction by transplantation of epidermal cell sheets onto the small intestine in an animal model.

Materials and Methods

Experimental animals

All experimental protocols were approved by the Animal Welfare Committee of Tokyo Women's Medical University. A female pig (20 kg) was injected intramuscularly with 0.04 mg/kg atropine and 15 mg/kg ketamine for premedication, and then injected intravenously with 2.5 mg/kg propofol. An endotracheal tube was inserted, and anesthesia was maintained using sevoflurane and nitrous oxide inhalation.

Cell culture

Epidermal cell sheets were prepared according to the method described previously.[12] Briefly, epidermal cells were isolated from the lower abdominal skin of the pig. The suspended cells were seeded in temperature-responsive cell culture inserts (CellSeed, Tokyo, Japan) and cultured in modified keratinocyte-conditioned medium for 2 weeks at 37°C in a humidified atmosphere containing 5%

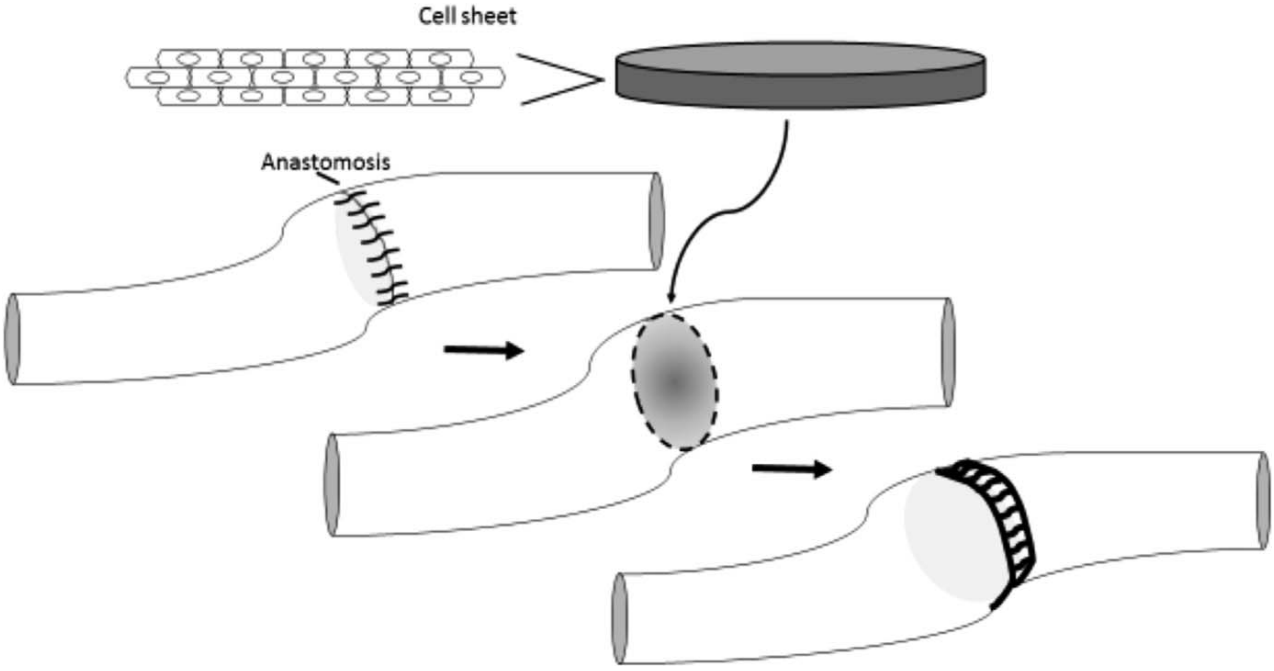

Fig. 1 This is new concept for strengthening of anastomosis.

CO_2. After culture for 2 weeks at 37°C, epidermal cells were transferred to another incubator set at 20°C for 20 minutes. The epidermal cell sheets were harvested noninvasively.

Operation

After laparotomy, the small intestine was cut 1.5 cm vertically at 6 points at 10-cm intervals (Fig. 2), and Gambee sutures were applied at 5-mm intervals (Fig. 3). The suture line was covered by the cell sheet with a sodium hyaluronate–based bioresorbable membrane (Seprafilm adhesion barrier; Genzyme, Cambridge, MA, USA) at 3 points on the intestine (Fig. 4a and 4b). The remaining suture lines were covered by sodium hyaluronate–based bioresorbable membranes. The abdomen was closed after a good fit was achieved with the transplanted cell sheets and sodium hyaluronate–based bioresorbable membranes. The small intestine with the transplanted cell sheets was resected 1 week after the operation.

Histology

The specimens were fixed in 4% paraformaldehyde (Wako Pure Chemicals, Osaka, Japan) and cut into paraffin-embedded sections 3 μm thick, which were stained with hematoxylin and eosin, azan, and Sirius red using conventional methods.

Results

All anastomoses were smooth on the mucosa and showed no narrowing (Figs. 5a–5c and 6a–6c). There were no findings of leakage after the operation. The controls showed more adhesions on the surface of the serosa compared with the transplantation group. The small intestine with cell sheet transplantation on the sutures showed considerable thickening (Fig. 6).

Histologic findings

Transplanted cells survived with a colony-like morphology on the surface of the serosa and subserosa on the small intestine (Fig. 7). Granulation tissue was recognized in both controls and transplanted specimens. However, the amount of granulation tissue was greater on the sutured small intestine with cell sheet transplantation compared with the controls. The area of connective tissue was measured using Sirius red staining in the vertical plain 5 mm from the suture line (Fig. 8). In controls, the average amount of connective tissue was 3.87 ± 1.72 mm^2 (5.70, 2.28, and 3.62 mm^2), whereas that in

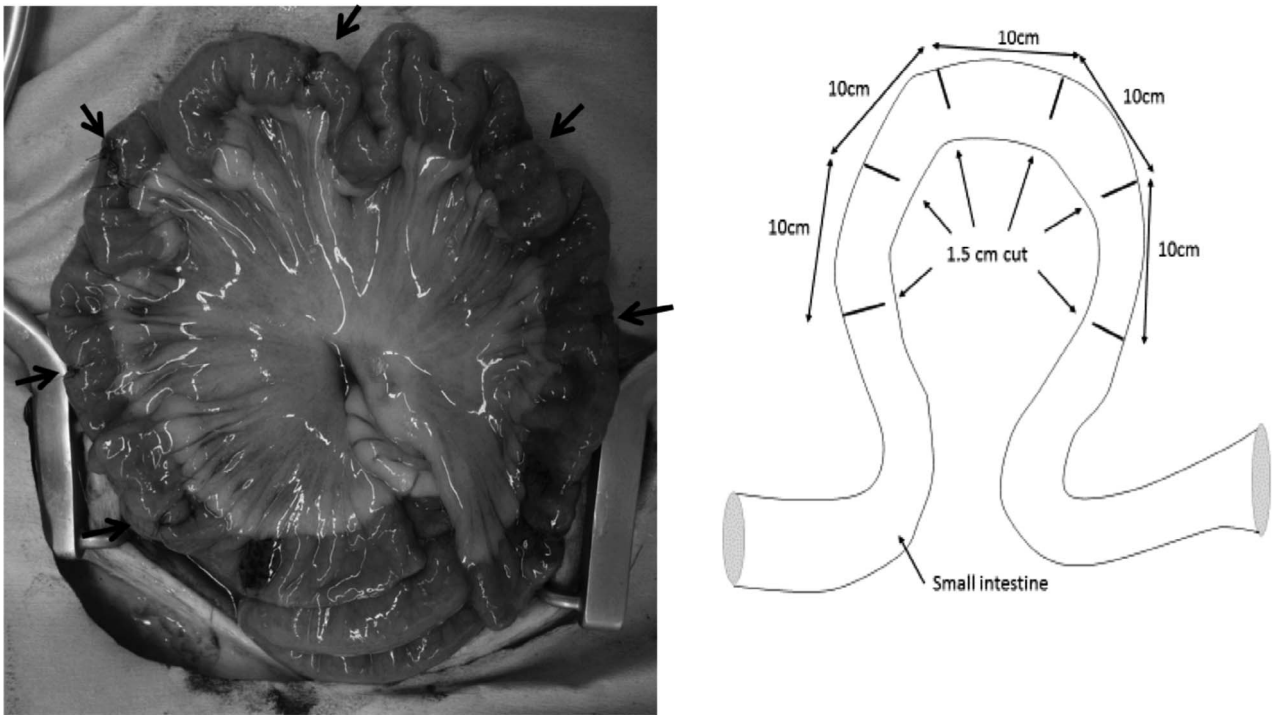

Fig. 2 The small intestine was cut at 6 points 1.5 cm vertically at 10-cm intervals.

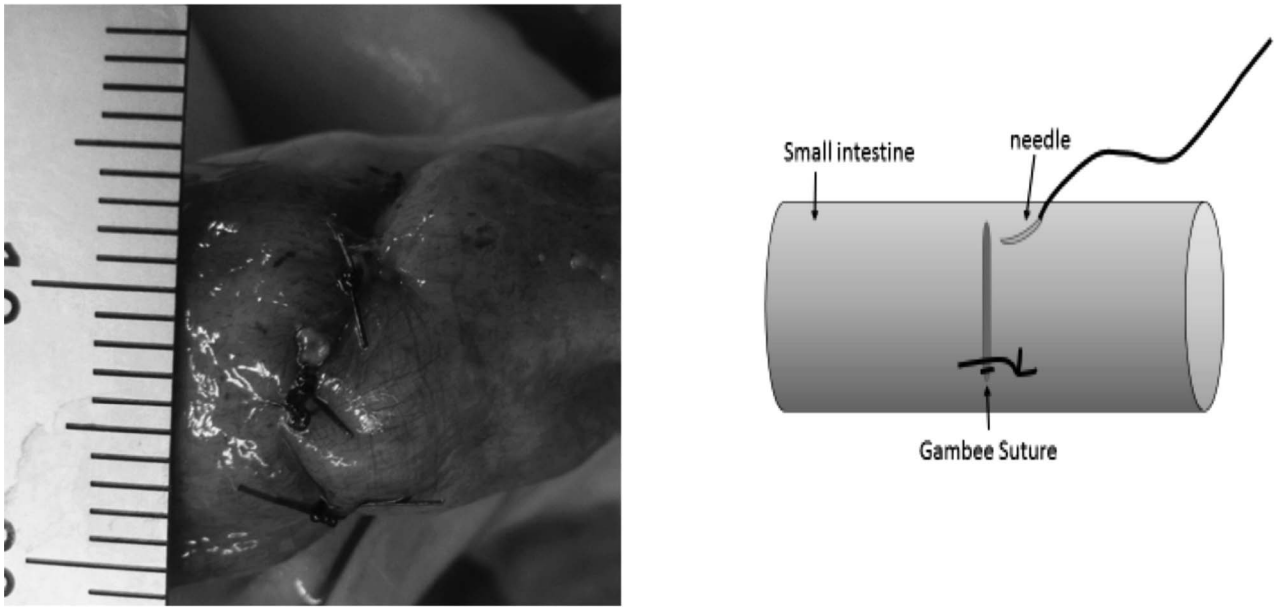

Fig. 3 Gambee sutures were implemented at 5-mm intervals.

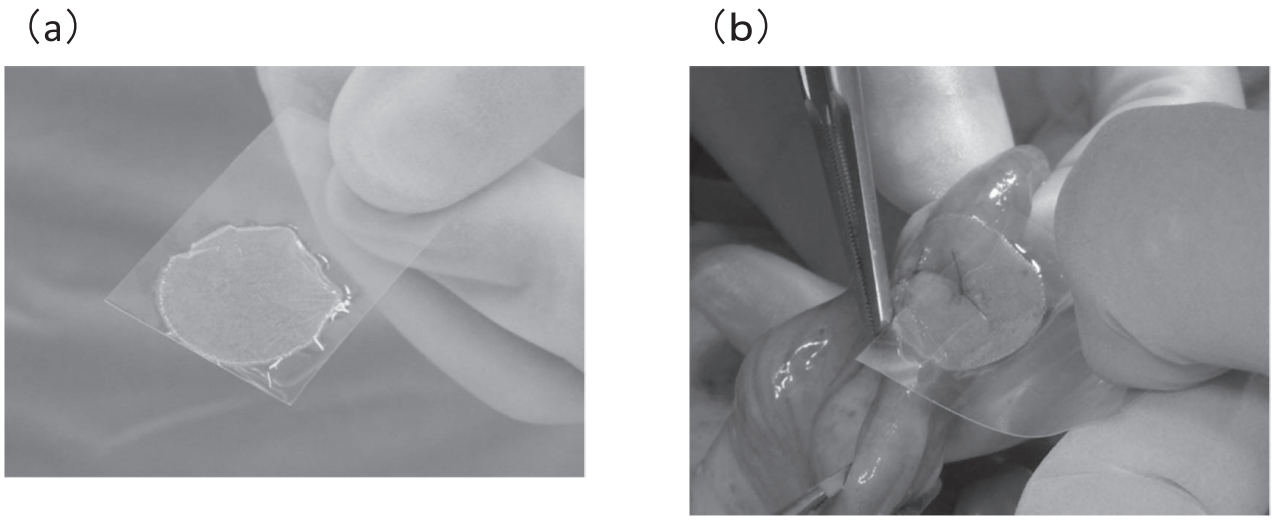

Fig. 4 Cell sheets are thin and fragile. Cell sheets are attached to the anastomotic site.

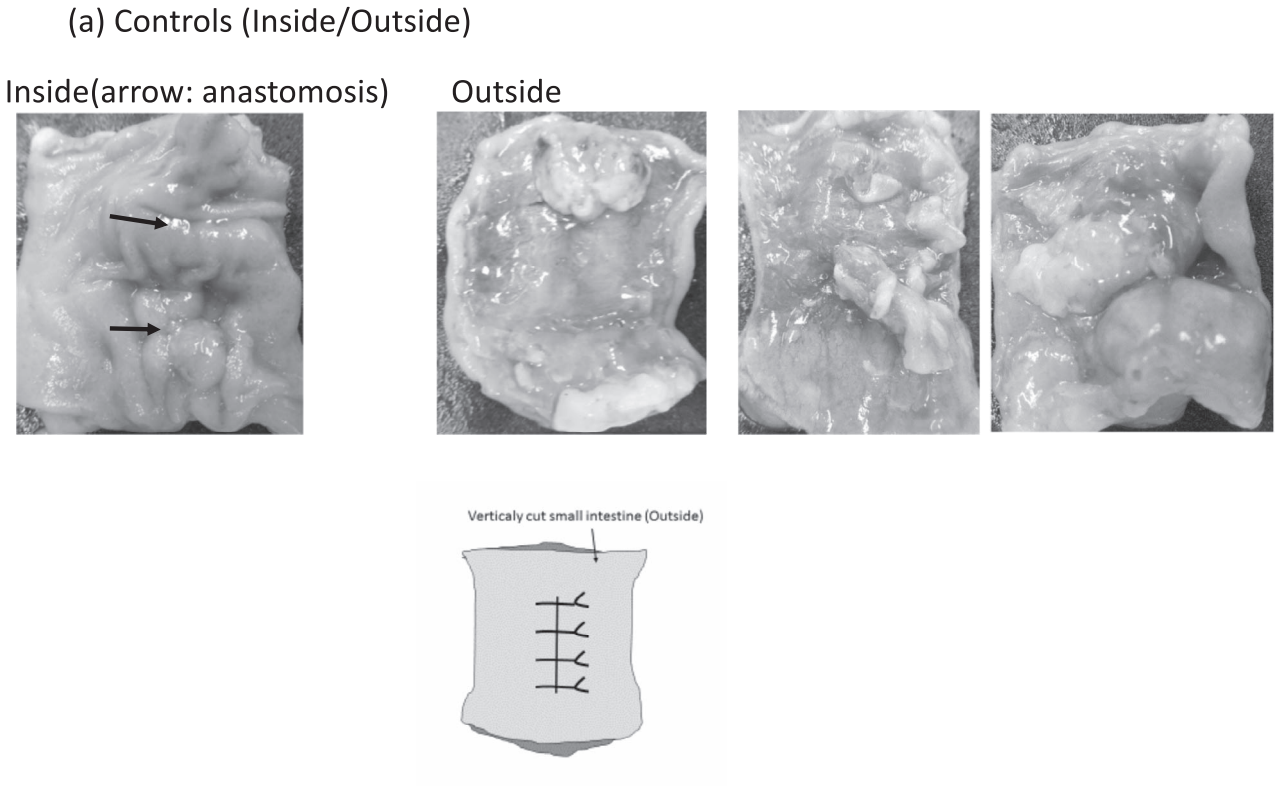

Fig. 5 All anastomoses were smooth on the mucosa and showed no narrowing. The shapes of cell sheets were confirmed outside of small intestine.

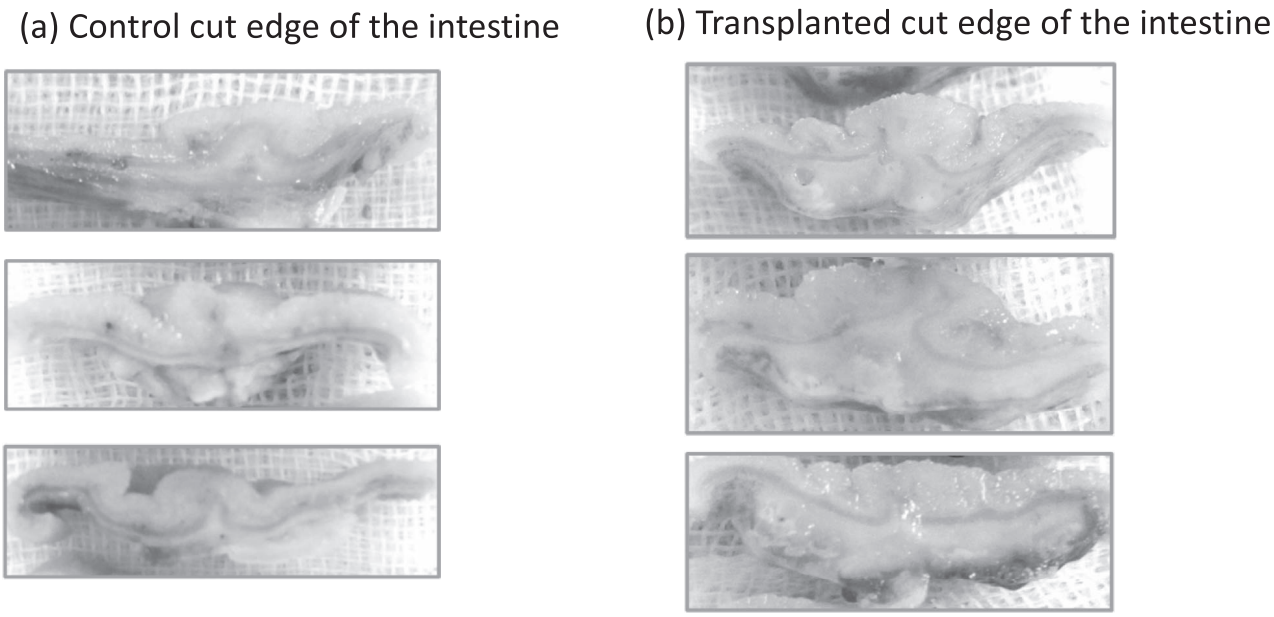

Fig. 6 The small intestine with cell sheet transplantation on the sutures showed considerable thickening.

transplanted specimens was 9.79 ± 1.25 mm^2 (11.1, 9.64, and 8.62 mm^2) (Table 1). Thus, the amount of the connective tissue was 2.53 times greater in transplanted specimens than in the controls.

Discussion

The results of the present study indicated that the cell sheet survived in the small intestine, and cell sheet transplantation on the anastomosis site caused thickening of the tissue. The amount of connective tissue was markedly increased 1 week after the operation in the transplanted specimens. Leakage is usually detected within 7 days after surgery, which corresponds to that in which increased connective tissue was seen in the transplanted specimens.

To prevent leakage from the sutures, it is desirable to strengthen the anastomotic site. The site of anastomosis in the rectum is fragile after chemoradiation therapy for rectal cancer. One clinical application of cell sheet transplantation involves sites of ischemic or strained anastomosis. The application of cell sheets to prevent leakage can be extended to anastomosis in many areas in gastroenterology, including esophagogastrostomy, pancreaticojejunostomy, and choledochojejunostomy.[13–17]

Table 1 Area of Sirius red staining within the anastomotic site 5 mm in width

	Connective tissue (mm^2)			
Cell sheets	Case 1	Case 2	Case 3	Average
Controls	5.70	2.28	3.62	3.87
Transplanted	11.10	9.64	8.62	9.79

Wound healing is a complex process that involves the coordinated functions of several cell types, including keratinocytes, fibroblasts, endothelial cells, macrophages, and platelets.[6,7,18] The process is also regulated by numerous growth factors, cytokines, and chemokines. In the present study, cell sheets were found to accelerate the healing process by an as yet unknown mechanism and promote wound healing of the anastomotic site. One possible reason for the effect of cell sheets on the healing process may involve the provision of matrix material, cytokines, and other regulatory molecules in the correct sequence.[5]

In the field of gastroenterology, transplantation of autologous oral mucosal epithelial cell sheets after endoscopic submucosal dissection (ESD) has advanced to the stage of a clinical study.[2] This previous report described the cell sheet attached to the inside of the esophagus. The transplant point is the distinction between this case and the previous report because the cell sheet was attached outside of the tract. In the small intestine, attachment to the mucosa is defective because of water inside the intestine, but that to the serosa

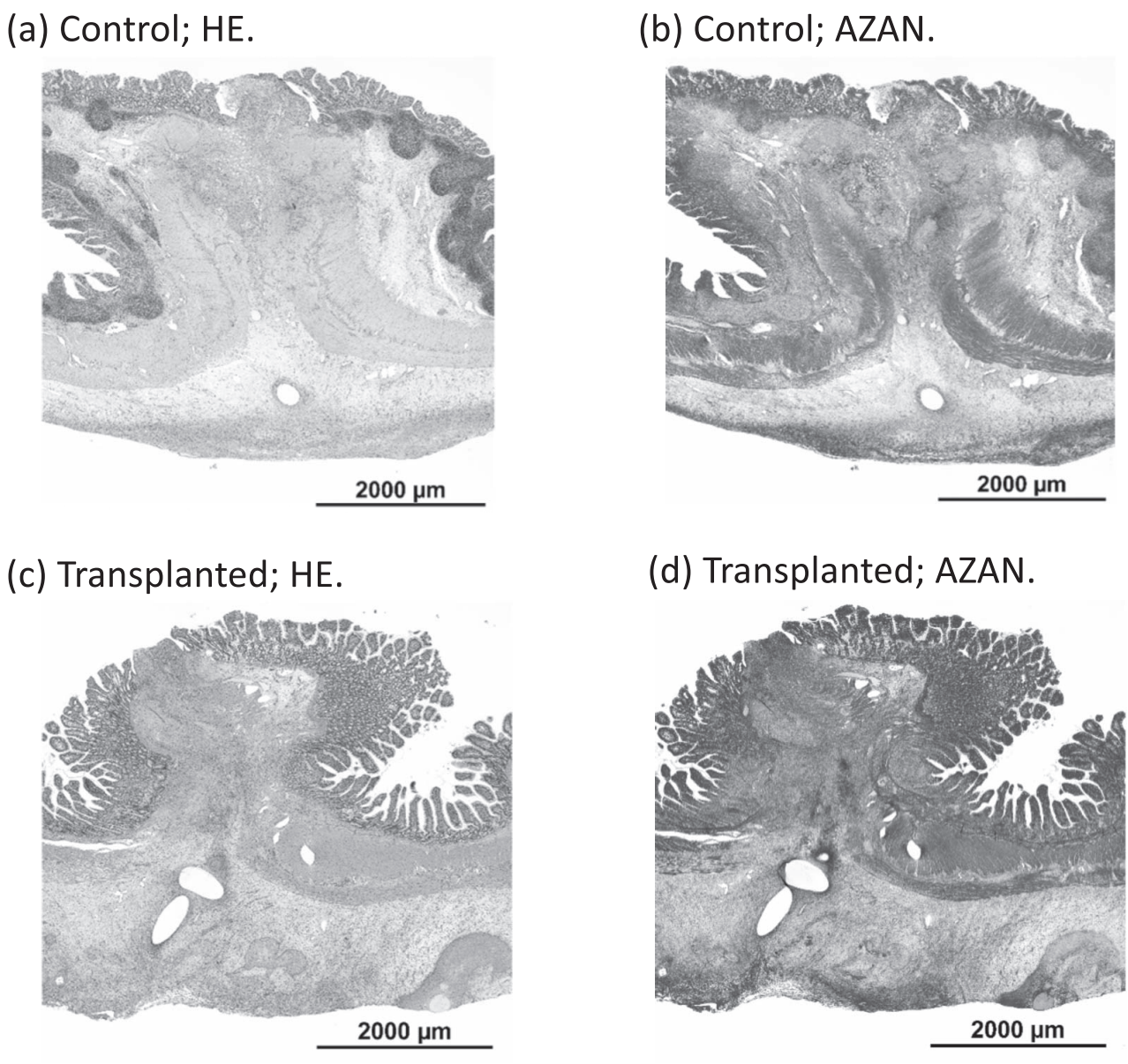

Fig. 7 Transplanted cells survived with a colony-like morphology on the surface of serosa and subserosa on the small intestine.

has greater retention ability to the serosa. In addition, external transplantation as described in the present report is reasonable because it is not possible to perform transplantation inside the intestine after suturing.

This is a unique paper about prevention of leakage using cell sheets. This study was performed in an experimental animal model and not in human patients. However, the results of the present study suggest that cell sheet transplantation may be applicable in clinical studies in the future. A new tissue engineering system and novel devices for use in transplantation have been developed in our institute.

Conclusion

Cell sheets applied to sutures in the small intestine survived and differentiated 1 week after transplantation. The amounts of connective tissue at the sites of anastomosis in the small intestine were increased in specimens with cell sheet transplantation compared with controls, and the area was about 2.53 times that in the controls. Further studies are

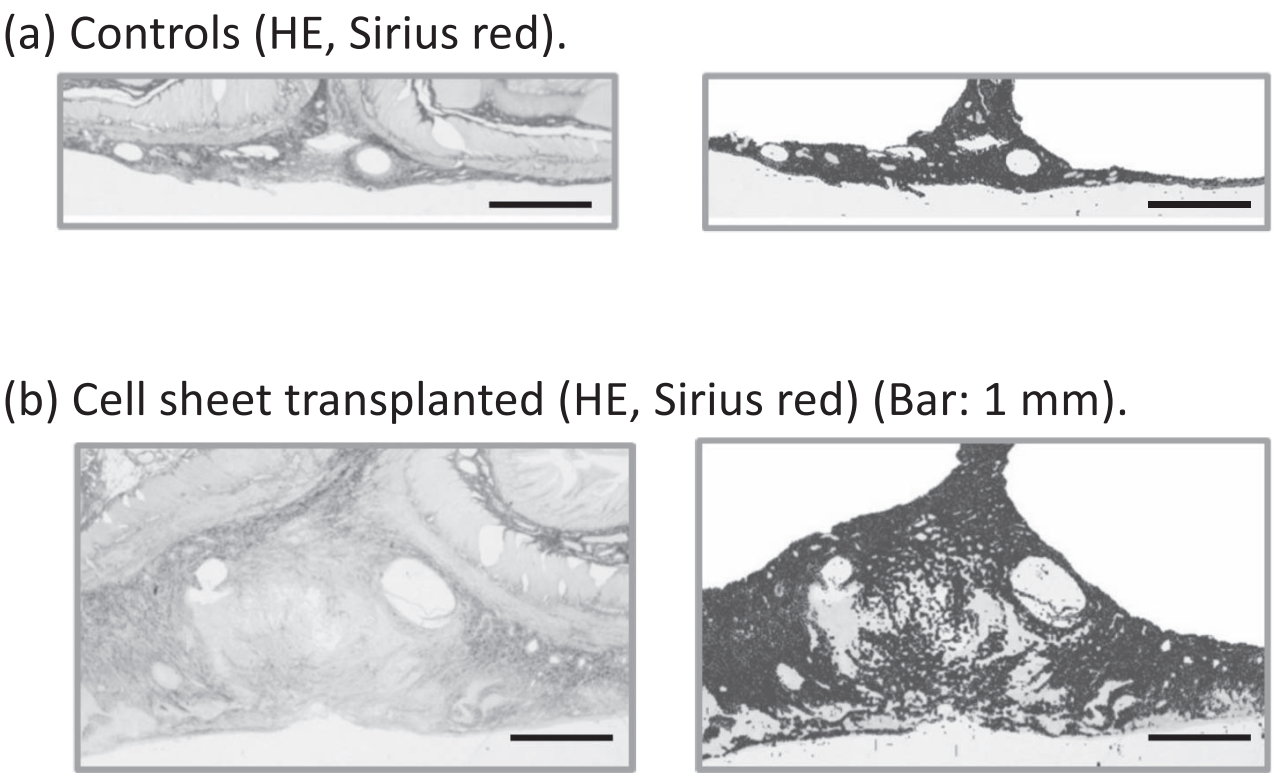

Fig. 8 The amount of granulation tissue was greater on the sutured small intestine with cell sheet transplantation compared with the controls.

required to identify the strength of anastomosis and substances that may enhance collagen synthesis and healing at sites of anastomosis.

Conflict of Interest

None of the authors has any commercial or personal associations that may pose a conflict of interest.

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