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End-to-End Anastomosis between Tissue-Engineered Intestine and Native Small Bowel

SATOSHI KAIHARA, M.D.,[1,2] STEPHEN KIM, M.D.,[1,3] MARK BENVENUTO, B.S.,[1] BYUNG-SOO KIM, Ph.D.,[4] DAVID J. MOONEY, Ph.D.,[4] KOICHI TANAKA, M.D.,[2] and JOSEPH P. VACANTI, M.D.[1,5]

ABSTRACT

The purpose of this study was to demonstrate the feasibility of end-to-end anastomosis between tissue-engineered intestine and native small bowel and to investigate the effect of this anastomosis on their growth. Microporous biodegradable polymer tubes were created from a fiber mesh of polyglycolic acid sprayed with 5% polylactic acid. Intestinal epithelial organoid units were harvested from neonatal Lewis rats and seeded onto polymers. These constructs were implanted into the omentum of adult Lewis rats. Three weeks after the implantation, the constructs ($n = 7$) were anastomosed to the native jejunum in an end-to-end fashion. Ten weeks after implantation, the tissue-engineered intestine was harvested. Four of 7 rats survived for 10 weeks and the overall patency rate of the anastomosis was 78% (11 of 14 anastomosis). The maximal length of the tissue-engineered intestine at week 3 and 10 was 1.80 ± 0.32 and 1.93 ± 0.39 cm (mean ± SD). Histologically, the tissue-engineered intestine was lined with a well-developed neomucosal layer that was continuous with the native intestine. We conclude that anastomosis between tissue-engineered intestine and native small bowel had a moderately high patency rate and had a positive effect on maintenance of the size of the neointestine and development of the neomucosa.

INTRODUCTION

SHORT BOWEL SYNDROME is a clinical condition characterized by malabsorption and malnutrition that occurs after massive small bowel resection.[1] With the development of total parenteral nutrition (TPN) and small bowel transplantation, many patients may survive for an extended period of time; however, these developments are often accompanied by a variety of complications.[2–5] Furthermore, organ donor shortage, especially in the pediatric population, continues to be a major problem in the transplantation field.[6,7]

Using the principles of tissue engineering, our laboratory has investigated the fabrication of a tissue-engineered intestine using synthetic biodegradable polymer scaffolds. Previous studies from this laboratory have

[1]Department of Surgery, Massachusetts General Hospital & Harvard Medical School, Boston, Massachusetts.
[2]Department of Transplantation Immunology, Graduate School of Medicine, Kyoto, Japan.
[3]Department of Surgery, University of Chicago, Chicago, Illinois.
[4]Department of Chemical Engineering, University of Michigan, Ann Arbor, Michigan.
[5]Department of Surgery, Massachusetts General Hospital, Boston, Massachusetts.
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demonstrated that fetal intestinal cells seeded on polymer tubes formed vascularized cysts with a well differentiated intestinal epithelium lining with mucous secretion.[8,9] Recently we also reported that intestinal epithelial organoid units seeded on biodegradable polymer scaffolds survive, vascularize, proliferate, and form cyst-like structures *in vivo*.[10] The cyst was lined with a neomucosal layer that expressed brush border enzymes, basement membrane proteins and electrophysiological properties similar to those of the normal small intestine.[11] We also reported that side-to-side anastomosis between the tissue-engineered intestine and native small bowel had no complications and kept a high patency rate.[12] This anastomosis had a positive effect on cyst growth and on development of the neomucosa.[13] In this study, we anastomosed the tissue-engineered intestine to native small bowel in an end-to-end fashion. Because the tissue engineered intestine will be placed in line with the native small bowel after the end-to-end anastomosis, all luminal contents will go through the neointestinal lumen, making it more physiological than a side-to-side anastomosis. The purpose of this study was to demonstrate the possibility of end-to-end anastomosis between tissue-engineered intestine and native small bowel and to investigate the effect of this anastomosis on the growth and development of the neointestine.

MATERIAL AND METHODS

Animals

Male and female 7-day-old neonatal Lewis ($RT1^l$) rats were used as donors for isolation of the epithelial organoid units. Adult male Lewis rats weighing 150–200 g were used as recipients. All animals were purchased from Charles River Laboratories (Wilmington, MA) and were housed in the Animal Research Facility of Children's Hospital, Boston, Massachusetts, in accordance with National Institutes of Health (NIH) guideline for the care of laboratory animals. Animals were maintained in a temperature-regulated environment on a 12-h light/dark cycle and given rat chow and tap water ad libitum. All recipients were inspected and their health assessed on a daily basis for the duration of this study after the tansplantation.

Polymer Fabrication

Highly porous, synthetic, biodegradable polymer tubes were created from sheets of a nonwoven mesh of polyglycolic acid (PGA) fibers (fiber diameter, 15 μm; mesh thickness, 2 mm; bulk density, 60 mg/cm^3; porosity, greater than 95%; mean pore size, 250 μm; Smith and Nephew, Heslington, York, UK, Fig. 1) as previously described.[14] In brief, a 15 mm $\times$ 10 mm of PGA mesh was wrapped around a Teflon cylinder to form a tube. The cylinder was then rotated at 20 rpm and solution of 5% (w/v) poly-L-lactic acid (PLLA) dissolved in chloroform was sprayed over it for 10 sec using an atomizer. After spraying, the solvent was evaporated and the polymer tubes were lyophilized for 48 h to remove residual solvent. The polymer tubes were sterilized with ethylene oxide and then coated with 200 μl of 0.31% collagen type I solution (Vitrogen 100, Collagen Corp., Palo Alto, CA), kept under UV light overnight. The collagen coated polymer tubes were washed three times with Hank's balanced salt solution (HBSS) Sigma Chemical Co., St. Louis, MO) before seeding.

Epithelial Organoid Unit Isolation and Seeding on Polymer

Intestinal epithelial organoid units were isolated as described by Evans et al.[15] In brief, the total small bowel was harvested from 7-day-old neonatal Lewis rats. They were lavaged with HBSS, slit open, and cut into small pieces. After several washings with HBSS, they were transferred into an enzyme solution containing 300 U/ml of collagenase XI (Sigma Chemical Co.) and 0.1 mg/ml of dispase (Boehringer Mannheim, Indianapolis, IN), and placed on an orbital shaker for 25 min at 25°C. After mechanical agitation they were further purified by centrifugation several times in Dulbecco's modified Eagle medium (DMEM, Gibco BRL, Gaithersburg, MD) with 2.5% fetal bovine serum (FBS) and 2% sorbitol. Isolated organoid units were resuspended in DMEM with 2.5% FBS and 2% sorbitol and seeded onto polymer at a density of 6.7–8.0 $\times$ 10^4 units/polymer. They were allowed to attach for 1 h before implantation.

Animal Operation

Adult male Lewis rats were used as recipients of the organoid unit-polymer constructs. Under methoxyflurane inhalational anesthesia, upper midline incision was performed and the abdominal cav-

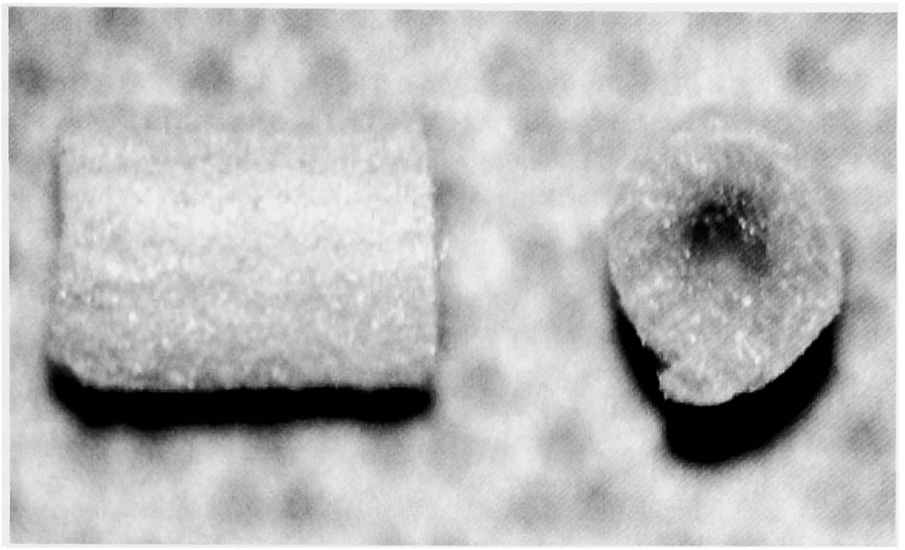

FIG. 1. PGA polymer tube.

ity was exposed. The unit-polymer constructs were wrapped with omentum and placed back in the abdominal cavity. Three weeks after the initial operation, the unit-polymer constructs formed a cystic structure. The rats with the cysts larger than 1.5 cm in length ($n = 7$) were selected for anastomosis. The cyst wall was roundly resected 5 mm in diameter on both sides and anastomosed to the native jejunum at 5 cm distal to the ligament of Treitz in an end-to-end fashion (Fig. 2). All rats were sacrificed 10 weeks after the initial operation and the tissue-engineered intestine was harvested for standard histological study.

Assessment

Cysts were measured 3 and 10 weeks after implantation by laparotomy. All specimens were stained with hematoxylin and eosin (H&E) for histological assessments. Morphometric analysis of the neomu-

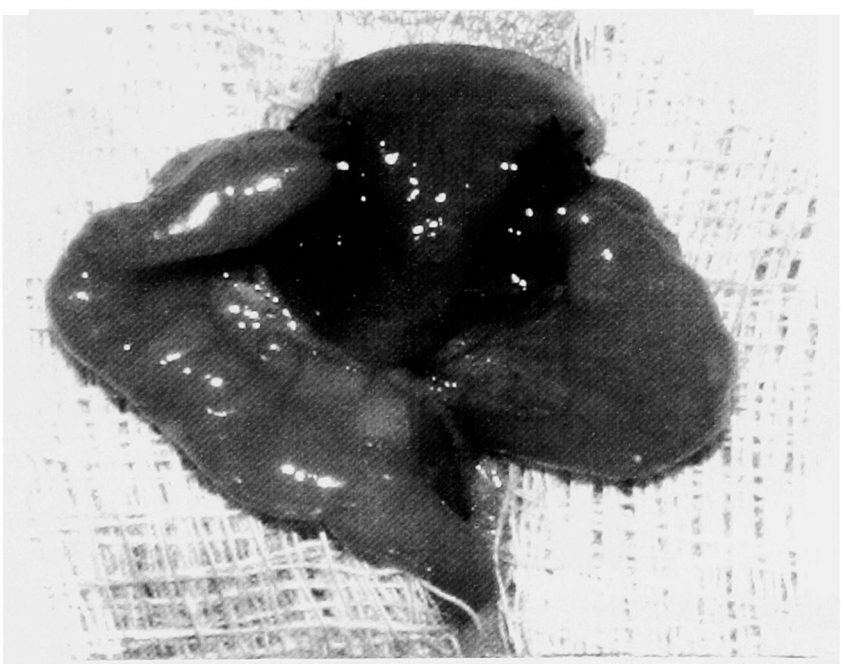

FIG. 2. Tissue-engineered intestine after anastomosis.

cosa was performed with a computer image analysis program (NIH image, version 1.59) using CCD-200E video camera (Videoscope Scientific Corp., Sterling, VA) mounted on a Leica inverted research microscope (Kramer Scientific Corp., Burlington, MA). Three 700-μm fields were randomly selected from each specimen (total, 12 fields) and villus number, villus height, and mucosal surface length were evaluated. Five 700-μm fields were also randomly selected from normal intestine and evaluated as control. They were expressed as mean villus number, mean villus height (μm), and mean surface length (mm) per 1 mm length.

Statistical Analysis

All data were expressed as mean ± SD. Statistic analysis was performed with Wilcoxon signed-rank test and two-group t test-unpaired. When the p value was less than 0.05, we judged it statistically significant.

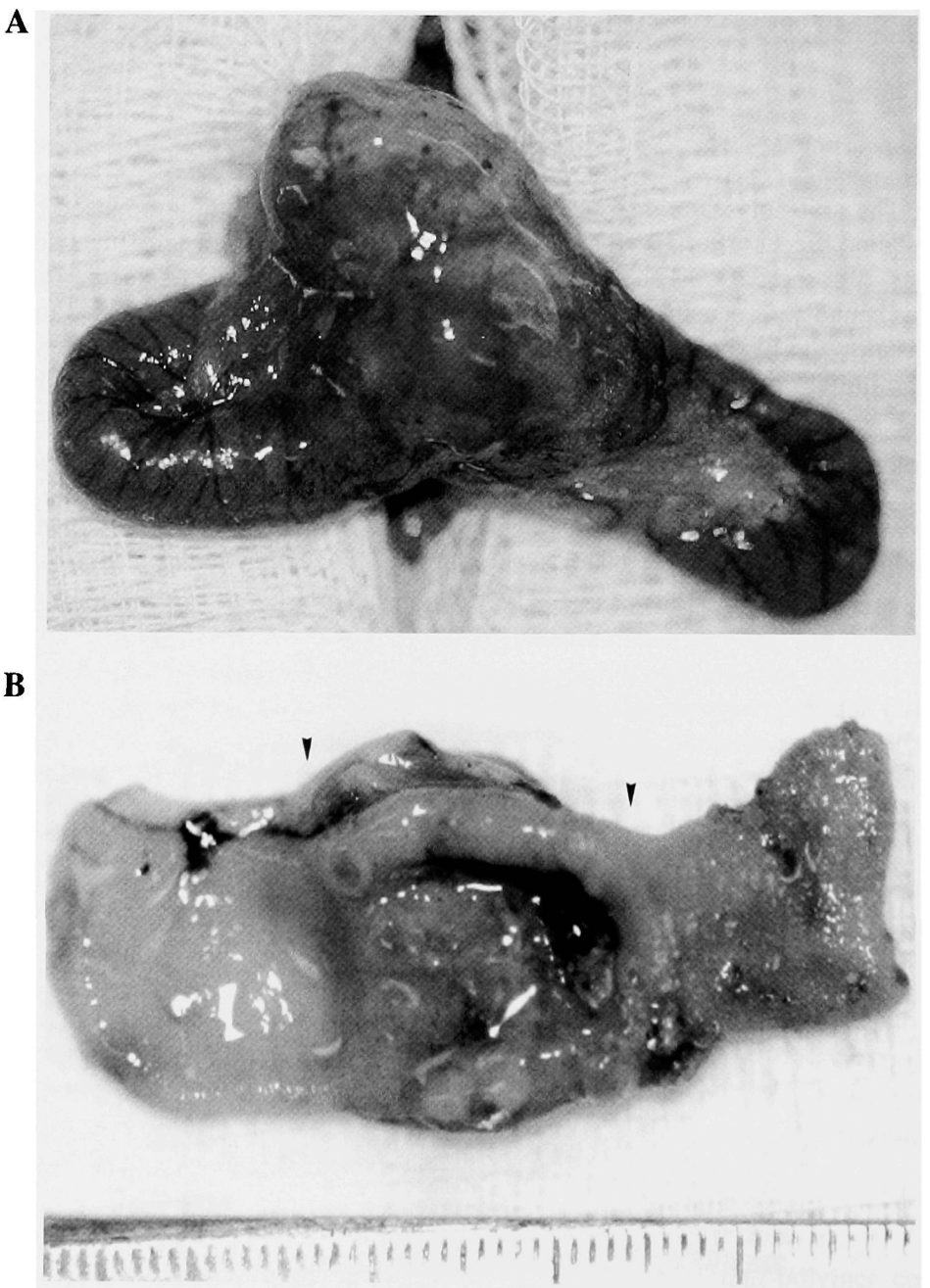

FIG. 3. (**A**) Anastomosed tissue-engineered intestine at 10 weeks. (**B**) Inner lumen of the anastomosis (arrowhead).

RESULTS

Four of seven rats survived for 10 weeks. Two died at day 11 and one died 19 days after their anastomosis. Rats increased their body weight significantly after their anastomosis (278.6 ± 3.8 g at anastomosis and 357.5 ± 26.3 g at sacrifice, mean ± SD). At autopsy, all of the rats had an obstruction on their distal anastomosis and the afferent intestine was remarkably dilated. The other four rats had no sign of stenosis or obstruction on their anastomosis at sacrifice. Overall patency rate of the anastomosis was 78% (11 of 14 anastomosis, Fig. 3A and 3B). The maximal length of the cyst at anastomosis and sacrifice was 1.80 ± 0.32 and 1.93 ± 0.39 cm, and there was no significant difference between them (Fig. 4). Histologically, the polymer had already been absorbed. The tissue-engineered intestine was lined with a well-developed neomucosal layer characterized by crypt-villus structure that was continuous to native intestine without any interruption (Fig. 5A and 5B). Morphometric analysis demonstrated that mean villus number, mean villus height, and mean surface length of tissue-engineered intestine was 12.6 ± 2.4 per mm, 246.2 ± 28.1 μm, and 6.17 ± 1.09 mm/mm, and those of normal intestine was 16.9 ± 0.6, 274.1 ± 23.3, and 9.80 ± 0.64, respectively. There were significant differences in mean villus number and mean surface length between them ($p < 0.01$ by two group t test-unpaired, Fig. 6). However, the obstructed anastomosis had a thick scar formation in the anastomotic site accompanied with inflammatory cell infiltration and the inner surface was not covered with a mucosal layer.

DISCUSSION

Our laboratory has been investigating the fabrication of tissue-engineered small intestine using biodegradable polymer scaffolds as a novel approach to treatment of short bowel syndrome. We have reported previously that the intestinal epithelial organoid units seeded on biodegradable polymer scaffolds formed vascularized cystic structures lined with a neomucosa surrounded by smooth muscle.[10] The neomucosa demonstrated expression of brush border enzymes, formation of a basement membrane and electrolyte transport functions similar to adult small intestine during *in vitro* experiments.[11] We also proved that a side-to-side anastomosis between tissue-engineered intestine and native small bowel had no complication with high

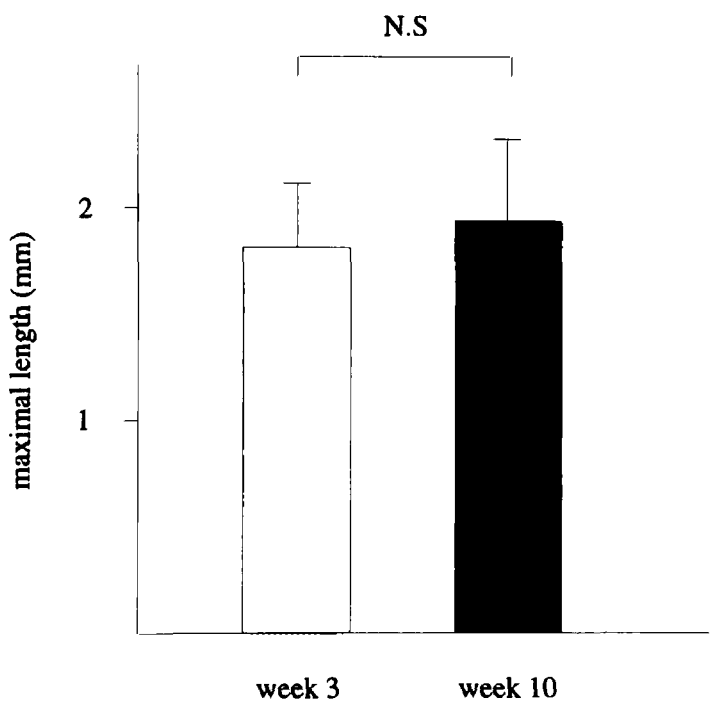

FIG. 4. Maximal length of tissue-engineered intestine at 3 and 10 weeks.

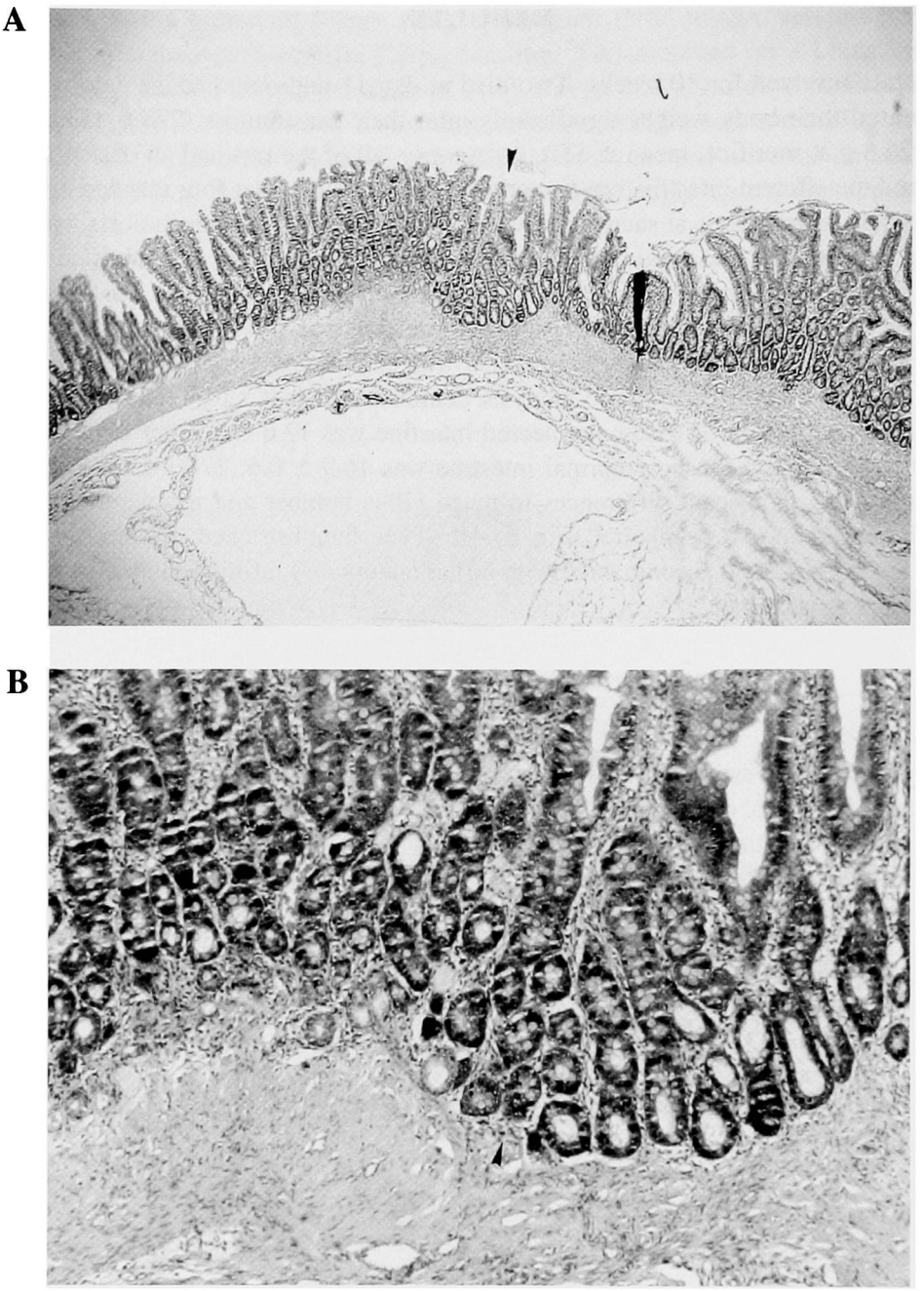

FIG. 5. Histology of the neomucosa and anastomsis (H&E staining, original magnification, (**A**) × 100; (**B**) × 250, arrowhead; anastomosis).

patency rate and had a positive effect on the growth of the cyst.[12,13] In this study, we demonstrated the possibility of end-to-end anastomosis between the tissue-engineered intestine and native small bowel because the neointestine was anastomosed to native intestine more physiologically in an end-to-end fashion than side-to-side.

In this study, tissue-engineered intestine maintained their size for 7 weeks after anastomosis. However, we had reported that tissue-engineered intestine gradually decreased their size without anastomosis.[13] Morphometric study also demonstrated that although mean villus number and mean surface length were still significantly lower than normal intestine, they were much greater compared to those without anastomosis in previous study.[13] These results suggested that end-to-end anastomosis had a positive effect on the maintenance of the structure and on the growth of neomucosa in the tissue-engineered intestine. As described

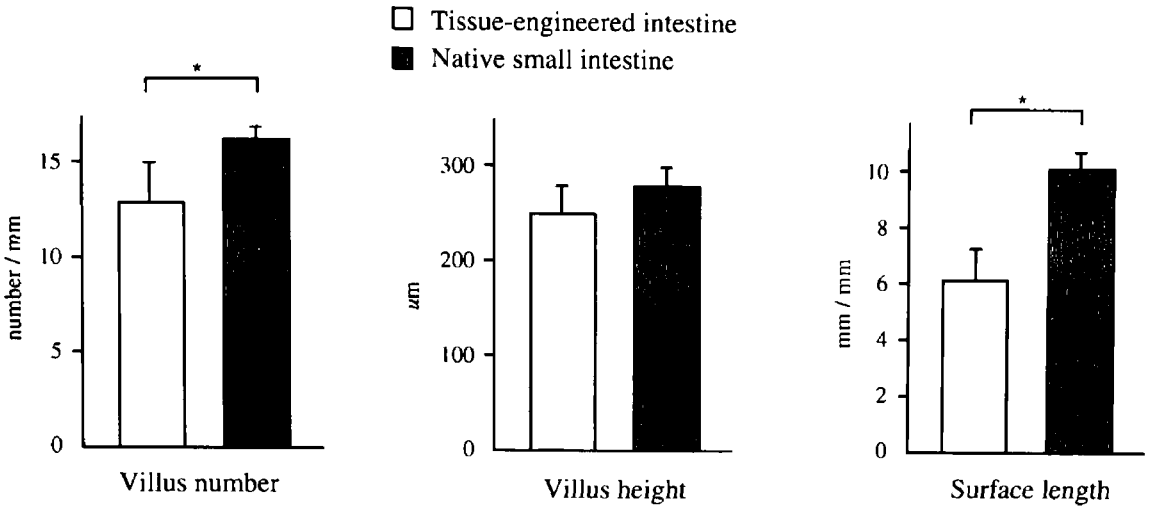

FIG. 6. Morphometric analysis of the mucosal layer in tissue-engineered intestine and native small bowel (*; $p < 0.01$ by two group t test, unpaired).

previously,[13] we postulated two possible mechanisms for this effect. First, the anastomosis decreased the inner pressure of tissue-engineered intestine by drainage of the luminal contents, and the lumen of the nonanastomosed neointestine was filled with retained luminal content and the neomucosa seemed to be compressed along the wall. Additionally, this high inner pressure may decrease the blood flow in the wall. After anastomosis the inner pressure may be decreased by the drainage of the luminal content and, therefore, the neomucosa may be able to grow. The second possibility is a stimulatory effect of the luminal contents on the neomucosa. It is well known that intestinal luminal contents have many important factors for mucosal development. Anastomosis allows access for the neomucosa to these stimulatory factors and may also have some beneficial effects compared to the nonanastomosed neointestine.

We have demonstrated in previous studies that side-to-side anastomosis also had a tropic effect on the size of tissue-engineered intestine and neomucosal development.[13] Which of the two kinds of anastomosis is a better way between tissue-engineered intestine and native small bowel? It is clear that end-to-end anastomosis is more physiological than side-to-side anastomosis because all luminal contents are passed through the lumen of the neointestine after end-to-end anastomosis. Histological study and morphometric analysis also demonstrated that end-to-end anastomosis had a more tropic effect on the neomucosal development compared to side-to-side anastomosis presumably because all luminal contents passed through the lumen of tissue-engineered intestine after end-to-end anastomosis.[13] However, end-to-end anastomosis had a much higher mortality rate than side-to-side because the obstruction at the anastomosis was always lethal after end-to-end anastomosis. Considering the mortality rate after the anastomosis, we believe that side-to-side anastomosis may be a preferable surgical technique than end-to-end anastomosis until we achieve a complete patency rate after anastomosis.

Side-to-side anastomosis had no surgical complications with 90% of patency rate[13]; however, the patency rate of end-to-end anastomosis was 78.6%. We speculate that the size of the anastomotic orifice was the reason for this difference. Because the tissue-engineered intestine was opened longitudinally for a side-to-side anastomosis, the anastomitic orifice was at least 10 mm; however, that of end-to-end anastomosis was 5 mm in diameter. We believe that a larger anastomotic orifice may be necessary to prevent obstruction.

In a previous study, we have reported that the neomucosa expressed brush border enzymes, basement membrane proteins and electrophysiologic properties during in vitro experiments.[11] We have also demonstrated the presence of nerve tissue, although it was not well organized, in the wall of the tissue-engineered intestine using immunohistochemical staining (data not shown). From these results, we speculated that this neointestine may have some function *in vivo* such as absorption and wall motility. Additional experiments are necessary to demonstrate this function.

In conclusion, we have demonstrated that end-to-end anastomosis between tissue-engineered intestine

and native small bowel had a moderately high patency rate, and had continuity in the mucosal layer. Furthermore, anastomosis had a trophic effect on cyst size and on the development of the neomucosa.

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Address reprint requests to:
Joseph P. Vacanti, M.D.
Department of Surgery
Massachusetts General Hospital
Harvard Medical School
55 Fruit Street, Warren 11
Boston, MA 02114

E-mail: jvacanti@partners.org

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