above the cephalic and the caudal anastomosis, and filling the tissue-engineered stomachs with saline solution through an inserted cannula until leakage was observed. The volume of the substitute of stomachs in the control group was measured by applying ligation just above the esophagojejunostomy and the Y-anastomosis, respectively, and filling the jejunum with saline solution through an inserted cannula until saline leaked from the jejunum.

For histological assessment, all specimens were fixed in 10% formalin and paraffin embedded. Serial sections (4 μm) were stained with hematoxylin and eosin (H&E) for histological assessment. Immunohistochemical staining was performed to identify proton pump (Clone 1H9, MBL Ltd., Nagoya, Japan) and gastrin (C-20, Santa Cruz Biotechnology, Santa Cruz, CA, USA), thereby identifying the existence of parietal cells and G-cells in the stomach mucosa. Labeled streptavidin–biotin system (DAKO, Carpinteria, CA, USA) was used for immunohistochemical staining. Blood samples were obtained 24 weeks after total gastrectomy to measure hemoglobin, total protein, and cholesterol concentrations for evaluating the nutrition status and anemia.

Statistical analysis

All data are expressed as mean ± standard deviation. Statistical analysis was performed using the standard one-way analysis of variance followed by the Bonferroni post hoc test. A P value of less than 0.05 was considered statistically significant.

RESULTS

The survival periods of the treatment group ($n = 15$) ranged from 7 to 176 days after the replacement surgery. Thirty-three percent of the recipients ($n = 5$) died within 2 weeks due to anastomotic leakage, and 47% ($n = 7$) also died between 2 and 4 weeks due to anastomotic stricture following minor leakage. Twenty percent of the treatment group ($n = 3$) survived the entire experimental period. In the control group, the survival rate for the entire experimental period was 100% ($n = 3$).

Figure 1a shows a macroscopic view of the tissue-engineered stomach before replacement surgery. Figure 1b shows a macroscopic view of the tissue-engineered stomach replacing the native stomach 24 weeks after surgery. The tissue-engineered stomach appears smaller after replacement. Although the volumes of the tissue-engineered stomach (8.0 ± 0.4 cc) and the substitute in the control group (5.7 ± 0.3 cc) are slightly reduced compared to that of the native stomach (10 cc), the upper gastrointestinal

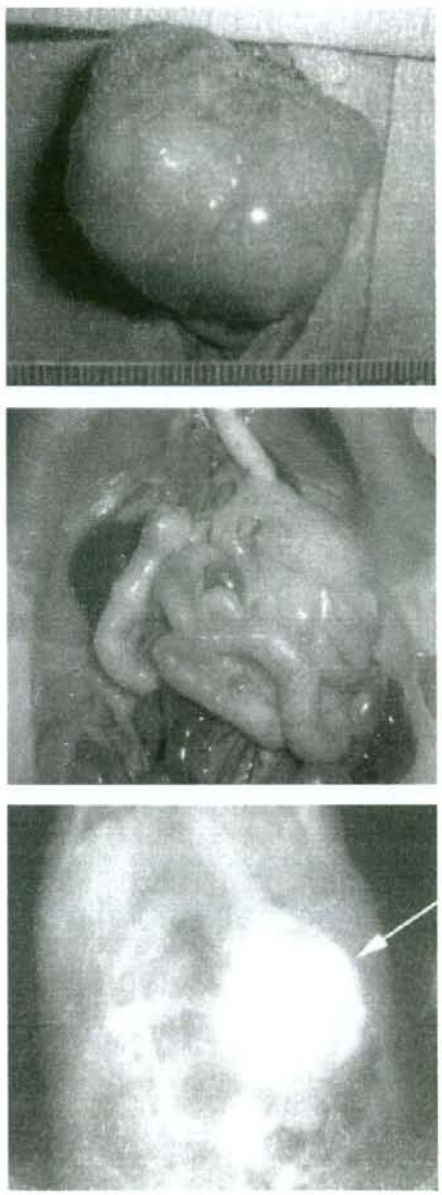

FIG. 1. (a) Macroscopic view of a tissue-engineered stomach before replacement. (b) Macroscopic view of a tissue-engineered stomach at 24 weeks after replacement. The liver has been removed to show the anastomosis site between the native esophagus and tissue-engineered stomach. (c) Upper gastrointestinal tract study at 10 min after barium injection. The arrow indicates the location of the tissue-engineered stomach.

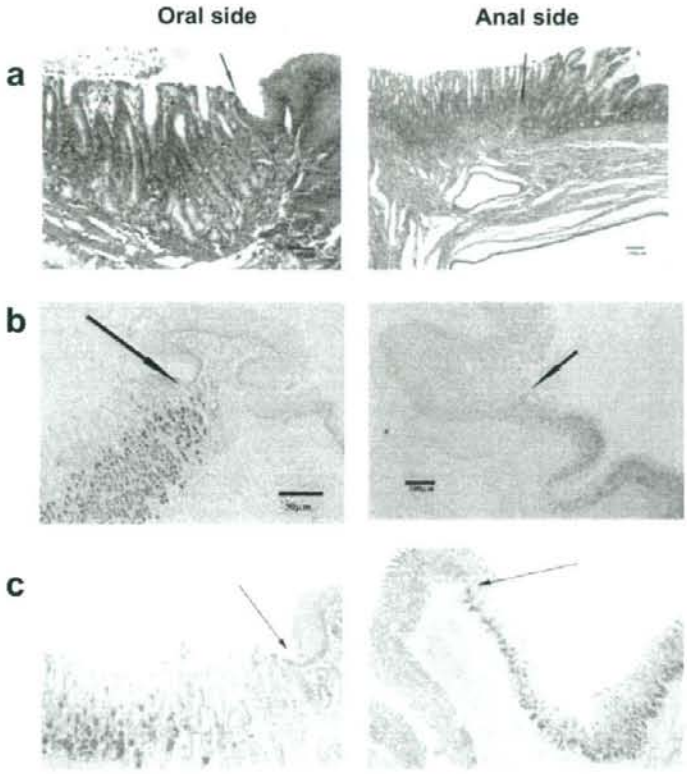

FIG. 2. The tissue-engineered stomach at 24 weeks after replacement. The arrow indicates the anastomosis site. (a) H&E stain (×40). (b) Immunohistochemical staining for proton pump (×100). (c) Immunohistochemical staining for gastrin (×100).

study revealed no evidence of bowel stenosis or obstruction at both anastomosis sites (Fig. 1c). The tissue-engineered stomach was filled with metabolites derived from both the stomach epithelium organoid units and the biodegradable PGA polymer, which appeared like the contents of an atheroma. It is conjectured that the contents played an important role in maintaining the lumen and volume of the tissue-engineered stomach.

The H&E-stained sections of the tissue-engineered stomachs at 24 weeks following replacement show well-developed vascularized tissue with a continuous neomucosa lining the lumen and stratified smooth muscle-like layers. There was no discontinuity in the mucosa and smooth muscle-like layers at both anastomosis sites (Fig. 2a).

Immunohistochemical staining for proton pump α-subunit showed the presence of parietal cells in the stomach mucosa (Fig. 2b). Immunohistochemical staining for gastrin was positive in the stomach mucosa, indicating the presence of G-cells (Fig. 2c). When viewed from the luminal surface, loops forming a capillary plexus were observed in scanning electron microscopy. Each capillary loop surrounded a gastric pit that extended through its center (data not shown).

Data for weight changes in the experimental groups are illustrated in Fig. 3. Although the intergroup difference was statistically insignificant between the treatment and control groups, the body weight of one rat ($n = 1$) in the treatment group was above the preoperative body weight at the end of the experimental period. The laboratory results are summarized in Table 1. The hemoglobin levels in the treatment group were significantly higher 24 weeks after total gastrectomy compared to the control group, although lower compared to untreated rats. No differences in total protein and cholesterol concentrations were observed in the blood samples at 24 weeks after total gastrectomy between the treatment and control groups. Both groups showed significantly lower total protein and cholesterol levels compared to untreated rats.

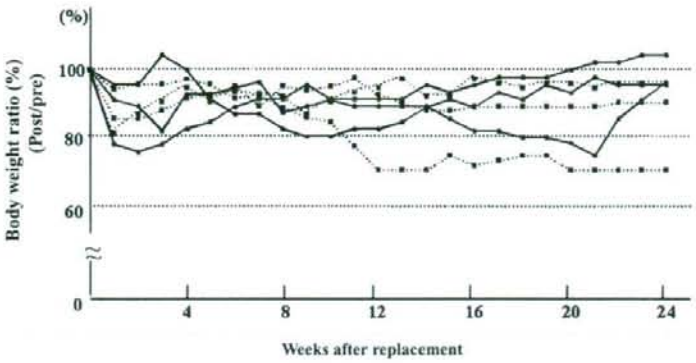

FIG. 3. Comparison of the body weight change in both groups over 24 weeks after replacement. The solid line denotes the weight change of the treatment group (tissue-engineered stomach). The symbols denote the control group (Roux-en-Y reconstruction).

DISCUSSION

Several studies on different reconstruction methods following a total gastrectomy have been carried out (4–8). Substitute stomachs used to date typically have no secretory functions due to the lack of gastric mucosal cells. Based on this requirement, the concept of a tissue-engineered stomach with a sufficient reservoir volume and secretory functions is appealing. In pathological findings, our results demonstrate a positive effect on the development of the epithelium. Because patients after a total gastrectomy have difficulty in absorbing vitamin B-12 from their alimentary tracts, they exhaust their vitamin B-12 reservoir within a few years after a total gastrectomy. This complicates pernicious anemia if the patients do not receive supplementary vitamin B-12. Our results indicate that the tissue-engineered stomach possesses the proton pump and G-cells comparable to the native stomach and thereby may improve refractory anemia following a total gastrectomy.

Body weight is usually well-correlated with quality of life. After total gastrectomies, only a few patients regain their weight prior to disease. The average rate of body weight loss ranges from 10 to 15% in the clinical experience. Although some studies suggest an effect of pouch reconstruction on body weight gain compared to a simple Roux-en-Y anastomosis, the effects remain clinically controversial (9–11). In the present study, one recipient rat ($n = 1$) in the treatment group reached its preoperative weight at 20 weeks. While this weight change does not show a clear advantage of the tissue-engineered stomach yet, a longer-term study may provide more insight. The hemoglobin level in the treatment group was higher compared to the control group, although still lower when compared to healthy rats. Total protein and cholesterol levels were also lower. Surgical stress may be a factor because it might severely impair the host defense, thereby exhausting the host from a nutritional viewpoint.

In the present study, three rats ($n = 3$) survived the entire experimental period of 24 weeks following the replacement surgery, while 12 rats ($n = 12$) died of leakage at anastomotic sites. It is conjectured that the leakage is most likely a consequence of the difference in wall thicknesses between the tissue-engineered stomach and the native jejunum, thereby requiring a complicated suturing technique. The shape of the tissue-engineered stomach and its fragility also may affect the difficulty for anastomoses. While the potential therapeutic effect of a tissue-engineered stomach is presently obscured by the low survival rate, the initial results are promising and further studies are warranted to assess the clinical potential of the tissue engineering approach.

TABLE 1. *Laboratory data for treatment group (tissue-engineered stomach), control group (Roux-en-Y reconstruction), and healthy group (untreated)*

Group	Hemoglobin (g/dL)	Total protein (g/dL)	Cholesterol (mg/dL)
Treatment group ($n = 3$)	9.4 ± 0.1*†	5.6 ± 0.1*	53.3 ± 2.5*
Control group ($n = 3$)	7.8 ± 0.6*	5.4 ± 0.1*	51.7 ± 1.8*
Healthy ($n = 3$)	14.0 ± 0.3	6.8 ± 0.1	67.0 ± 3.2

* $P < 0.01$ versus healthy group.
† $P < 0.05$ versus control group.

CONCLUSION

In conclusion, the present study demonstrates that a tissue-engineered stomach has the ability to act as a food reservoir and possesses secretory functions comparable to a native stomach. Gastric tissue engineering may have the potential to provide a positive effect on improvements of anemia and body weight loss following total gastrectomy.

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