Omentum pediculed musculo-peritoneal flap (OPMP) for growing intestinal neomucosa in a rat model

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Summary

Background/Purpose: To construct a musculo-peritoneal flap adequately vascularised through attached omentum and to evaluate whether this flap is functional for growing new intestinal mucosa.

Methods: 24 rats underwent two surgical procedures. 1) A musculo-peritoneal flap was prepared, omentum was fixed onto the muscular side and a silicon graft was placed on the peritoneal side. 2) After one month the so constructed OPMP flap was mobilised and attached to the jejunum. Animals were harvested at 2, 4 and 8 months for macroscopic and microscopic evaluation.

Results: One rat died at 7 months due to intestinal volvulus. In the remainder the circumference of the patched intestine significantly increased compared to that of normal intestine (p <0.001).

The flaps were markedly contracted in the 4 month rats (p <0.01). All flaps were completely covered by neomucosa except in the 2 month rats. Crypt depth and crypt density of the neomucosa was significantly lower in 2 month rats compared to other groups (p <0.05). Similarly neomucosal villous height was markedly low in the 2 month rats (p <0.01).

Conclusions: To date, we have found no reports of small bowel patching using this technique. In this context, use of OPMP flap is likely to be useful for increasing intestinal surface area.

Key words: omentum; abdominal wall muscle; peritoneum; intestinal neomucosa

Introduction

Several medical and surgical procedures have been designed to increase small bowel surface or to slow intestinal transit in patients following massive bowel resection [1, 2]. Recently methods for expanding the small bowel absorptive area by intestinal transplantation, bowel lengthening, omental flaps and growing new intestinal mucosa have been investigated [2]. In experimental models, patches including serosal surface of adjacent colon, prosthetic materials and abdominal wall muscle have been commonly used for growth of intestinal neomucosa [3–5]. However we have found no reports of small bowel neomucosa growing on vascularised pedicle flaps of abdominal wall musculature and peritoneum based on the omentum. In this study, we have attempted to create an adequately vascularised omentum pedicled musculo-peritoneal (OPMP) flap and to test the ability of this flap to grow intestinal neomucosa.

Materials and methods

The study was initiated after approval by the Animal Investigation Committee of Akdeniz University School of Medicine had been obtained. 24 female Swiss-Albino rats, weighing between 210 and 240 grams, were used. All surgical procedures were performed after an overnight fast using sterile techniques. Anaesthesia was administered intramuscularly with xylazine (10 mg/kg) and ketamine (50 mg/kg).
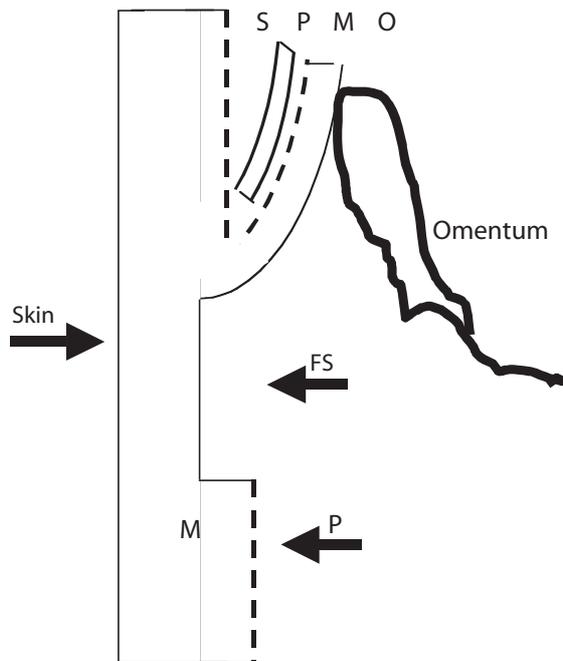
Surgical technique

All rats underwent two operations.

1) Preparation of an OPMP flap: During the first operation an OPMP flap was created. A large midline incision was made. A 2×1-cm flap containing deep portions of rectus, transverse muscle and peritoneum was fashioned from the lateral abdominal wall at a distance of about one centimetre from the incision on the base of its superior edge. The muscle layer of the flap was kept as thin as possible in order to ease omental vascularisation. To prevent contraction, a Dacron reinforced silicon graft of equal size to the flap was attached to the peritoneal side using four corner 5-0 silk sutures. The flap was rotated up through 180°s and fixed just inferior to the wound edge so that the muscular layer was facing the abdominal cavity. As a final step omental leaves were sutured with 5-0 silk to the muscular layer of the flap (Fig. 1). The abdominal wall was closed with continuous 4-0 silk closure. All rats were kept in standard laboratory conditions for one month.

2) Patching: One month after this procedure the animals were prepared for the second stage, namely patching. Following midline laparotomy, the OPMP flap was separated from the abdominal wall avoiding any injury to the omental pedicle (Fig. 2A). The silicon graft was removed from the peritoneal side and the flap was measured again to confirm its 2×1 cm size (Fig. 2B). A segment of jejunum located approximately 25 cm distal to the ligament of Treitz was opened for 2 cm along the antimesenteric edge. The OPMP flap was anastomosed to the open bowel with a continuous single layer closure of 6-0 polyglactin 910 sutures. The midline incision was closed with continuous 4-0 silk. The rats were given subcutaneous injections of saline solution on the first postoperative day. Water and food was provided ad libitum on the second and third postoperative days, respectively. The animals were followed daily for activity, evidence of poor feeding, weight loss and constipation.

Macroscopic and microscopic examination

The patched intestines were harvested at 2 months (8 animals), 4 months (8 animals) and 8 months (7 animals). The circumference of the patched intestine and that of the normal jejunum was measured. The tissue specimens were fixed in 10% formalin, embedded in paraffin and stained with Haematoxylin and Eosin (HE). Image analysis was performed using a SAMBA 2005 image processor (Alcatel-TITN, Grenoble, France). Two observers performed morphometric evaluation without knowing the harvesting period of the rats. Villous height, crypt depth and crypt density were determined from axially oriented normal mucosa and neomucosa on 5 μm thick tissue sections. Villous height and crypt depth were evaluated at ×250 and crypt density at ×400 magnifications, respectively.

Statistical analysis

All data were expressed as means ± standard deviation. Results were subjected to one-way analysis of variance for repeated measure and statistical significance was determined by LSD test to compare values among groups (SPSS 9.0 for Windows). Differences were considered significant when $p < 0.05$.
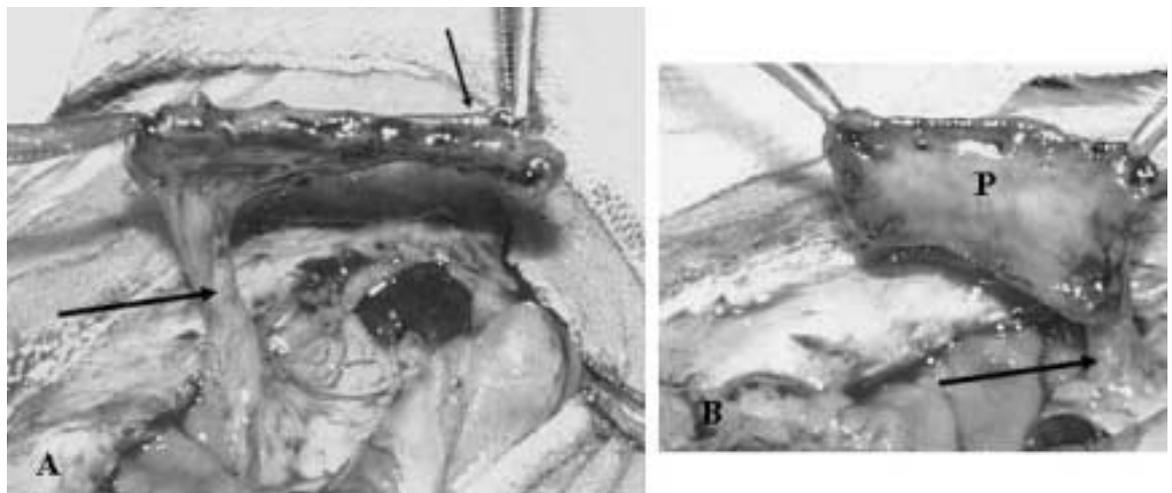
Figure 3

The OPMP flap was completely covered by neomucosa in rat harvested at 8 months.
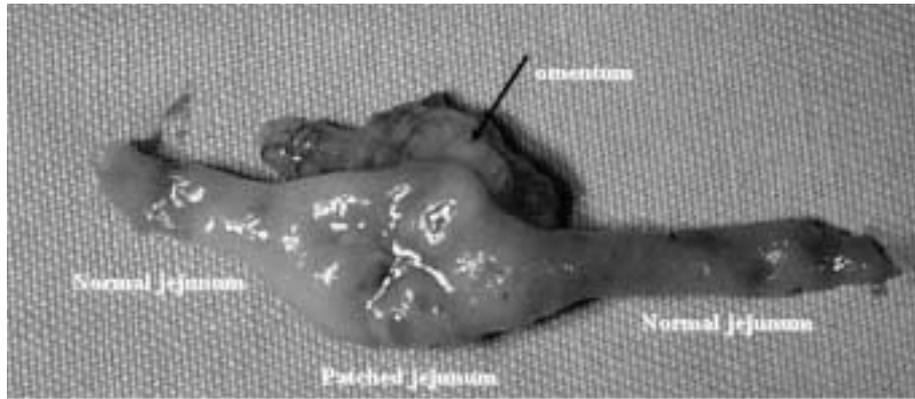
Results

Twenty three of 24 animals (95.8%) survived the duration of the study. One animal died due to intestinal volvulus in the seventh month after patching. All remaining animals gained weight and demonstrated normal behaviour and feeding activity.

During the second laparotomy the flap site of 2×1 cm on the abdominal wall was found to be completely covered by peritoneum. During harvesting the anastomosis was intact and there was no sign of intestinal dilatation, anastomotic leak or stenosis. Intraperitoneal adhesions were detected in two rats at 2 months and one rat at 4 months.

The border of the omental pedicle on the antimesenteric surface was considered to be the margin of the original anastomosis between OPMP flap and jejunum. On macroscopic examination of the animals sacrificed at 2 months, fibrotic tissue, which was progressively covered by neomucosa spreading from the lateral margin to the centre, was present on the flaps. However macroscopic examination demonstrated that the OPMP flaps were completely covered by neomucosa in rats terminated at 4 and 8 months (Fig. 3), except in one 4 month rat in which the flap showed ulceration and granulation tissue.

The circumference of the patched bowel was 11.6 ± 0.5 mm in 2 month rats, 10.5 ± 0.7 mm in 4 month rats and 11.4 ± 0.7 mm in 8 month rats (Fig. 4). The bowel was found to be significantly dilated in 2 and 8 month rats compared to 4 month rats ($p < 0.05$). The adjacent normal jejunum had a circumference of 6 ± 0.7 mm, 5.6 ± 0.7 mm and 6.1 ± 0.6 mm in 2, 4 and 8 month rats, respectively. The jejunal segment at the site of patching was significantly larger than the normal jejunum in all animals ($p < 0.001$). The OPMP flap was found to be markedly contracted in 4 month rats compared to

Figure 4

Circumference significantly increased in the patched bowel in contrast to normal bowel; ‡ ($p < 0.001$): I, II, III vs. I, II, III. However, circumference of the patched bowel was markedly lower in 4 month rats compared to the other rats, * ($p < 0.05$): II vs. I, III.
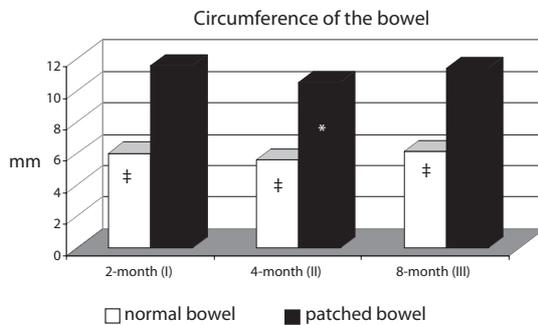
Figure 5

Two month specimen. Photomicrograph showing single layer of columnar epithelial cells in the central area of the flap. Irregular and poorly developed villi were observed at the lateral margins (HE ×10).
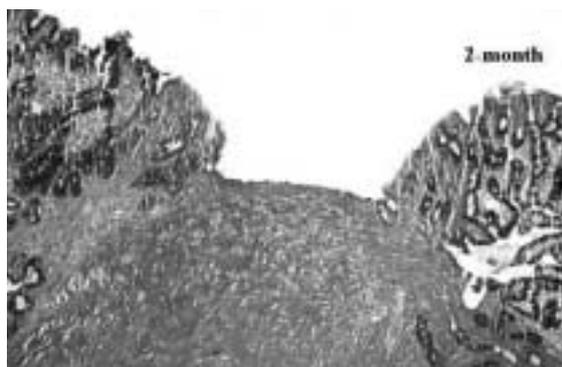
Figure 6

Four month specimen. Flap was fully covered by neomucosa consisting of irregular branching villi, which overlaid smooth muscle and fibrous tissue. Arrow shows omentum attaching to the flap (HE ×10).
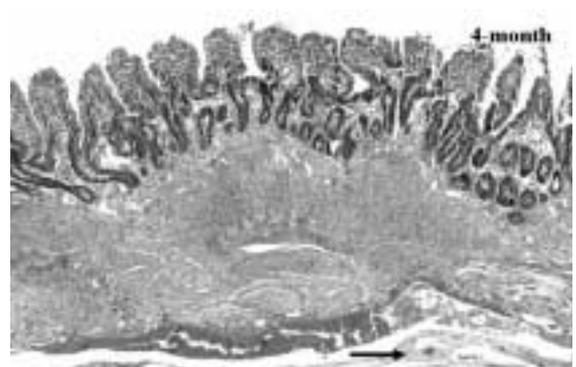
2 and 8-month rats (32.9% ± 2.3 vs. 27.4% ± 2.4 and 29.3% ± 2.9; p <0.01).

Histological examination revealed that the surface of the flap was epithelialised at 2 months. Neomucosa consisted of a single layer of columnar epithelial cells, especially in the central area. At the lateral margins some irregular, poorly developed villi were present (Fig. 5). At 4 months, irregularly branching villi at the base of the lamina propria and muscularis mucosa overlying smooth muscle and fibrous tissue were prevalent (Fig. 6). Their maturation was higher at the lateral margins. By 8 months a well-developed neomucosal layer continuous with the native intestine was observed. This layer exhibited well-formed mature crypts and villi and contained goblet cells (Fig. 7).

Histopathological parameters including villous height, crypt depth and crypt density are summarised in Table 1. Villous height was significantly lower in 2 month rats compared to other groups (p <0.01). There was no difference between the villous height of the neomucosa and the normal mucosa in 4 month and 8 month rats.

Crypt depth of the neomucosa was markedly lower in 2 month rats than in 8 month rats (68.7 ± 9.7 μm vs. 81.7 ± 6.8 μm, p <0.05). In addition 2 month rats had significantly smaller neomucosal crypt depth compared to normal mucosa (68.7 ± 9.7 μm vs. 82.5 ± 11.4 μm, p <0.05). However crypt depth of the neomucosa was similar to the normal mucosa in 4 and 8 month rats.

Crypt density of the neomucosa was markedly lower in 2 month rats than in 8 month rats (181.8 ± 30.7 vs. 207.8 ± 19.05 mm²/density, p <0.05). Additionally 2 month rats had significantly smaller neomucosal crypt density than normal mucosa (181.8 ± 30.7 vs. 210.1 ± 22.3 mm²/density, p <0.05). The crypt density of the neomucosa was similar to the normal mucosa in 4 and 8 month rats.

Figure 7

Eight month specimen. Neomucosa has well-developed regular villi and mature crypts, and is similar to the normal small bowel mucosa.
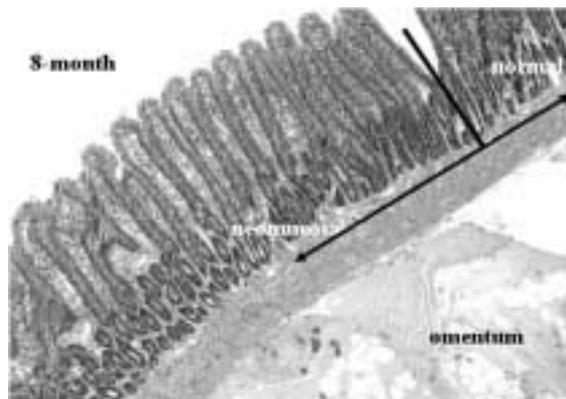
Table 1

Comparison of the histopathological parameters.

	Normal mucosa			Neomucosa		
	2 month (I)	4 month (II)	8 month (III)	2 month (I)	4 month (II)	8 month (III)
Villous height	457.2 ± 63.3	461.9 ± 52.7	466.9 ± 42.7	352.5 ± 25.7*	448.2 ± 59.4	469.7 ± 49.8
Crypt depth	82.5 ± 11.4	78.5 ± 9.5	80.6 ± 9.6	68.7 ± 9.7‡	76.4 ± 9.5	81.7 ± 6.8
Crypt density	210.1 ± 22.3	203.5 ± 11.3	205.1 ± 12.8	181.8 ± 30.7¶	198 ± 9.7	207.8 ± 19.05

* (p <0.01): I vs. II, III.   ‡ (p <0.05): I vs. III; ‡ (p <0.05): I vs. I.   ¶ (p <0.05): I vs. III; ¶ (p <0.05): I vs. I.

Discussion

Although the incidence of spontaneous adaptation in cases of short bowel syndrome is approximately 75%, refractory cases following massive bowel resection require total parenteral nutrition and surgical therapy [6]. Surgical techniques basically aim to prolong intestinal transit and increase absorptive surface area. Apart from Bianchi and Kimura's autologous gut lengthening technique, growing new intestinal mucosa as a means of increasing intestinal absorptive capacity is being investigated as a probable solution to the short bowel syndrome [3, 7–9]. To date, growth of intestinal neomucosa has successfully been detected in colon serosa, abdominal wall pedicle flaps, some prosthetic materials, colon segments stripped of mucosa, human amniotic membrane and gastric homologous acellular matrix [4, 9–11]. Thompson et al. [4] stated that both colon serosa and abdominal wall musculature could support the growth of functioning neomucosa. The growth rate and the functional quality of the neomucosa were similar in both groups. Lillemoe et al. [12] showed growing rabbit ileal mucosa on vascularised pedicle flaps of abdominal wall musculature derived from the inferior epigastric artery and that the neomucosa was similar to normal mucosa using microscopic and electrophysiological evaluation. Furthermore, Yin and Dadoukis et al. [13] suggested in experimental models that pedicle grafts of peritoneum and transversalis muscle may be useful in the treatment of patients with large defects in the wall of the duodenum. Excluding these patching methods, the use of prosthetic patches has not been shown to be useful in the management of short

bowel syndrome. Thompson et al observed that prosthetic materials such as Dacron, polyglicolic acid mesh and politetrafluoroethylene patches were either extruded or dissolved within a functional neomucosa. To the best of our knowledge a pedicled flap of abdominal wall musculature and peritoneum derived from omental leaves for growing intestinal neomucosa has not been previously described.

Omentum possesses the mechanical functions of the mesentery, which is the fixation of the viscera and the transmission of the vascular supply [14]. Furthermore omentum has a special motility that allows it to detect and solve problems occurring within the peritoneal cavity and to develop new blood vessels rapidly. This unique ability of the omentum has resulted in its utilisation in various treatment techniques, such as reconstruction of the head and neck, chest wall defects, irradiated wounds, reinforcement of intestinal anastomosis, vaginal reconstruction and bowel elongation by omentoenteropexy [16–19]. Rand et al. [1] showed that omentoenteropexy alone with no bowel lengthening technique could enhance the intestinal absorptive capacity. In the present study we demonstrated that an OPMP flap was capable of growing intestinal neomucosa in a rat model. However, the investigators experienced serious difficulties during the creation of the flap. Prior to the use of a Dacron reinforced silicon graft, in order to prevent adhesion of the flap to the abdominal wall or the disappearance of the flap, we could not obtain any OPMP flaps. After this successful modification, the observed mortality rate of the rats was very low. Lillemoe et al. [12] reported a 30% mortality rate in rabbits with vascularised abdominal wall pedicle flaps used to grow small bowel neomucosa. Most experimental studies showed complete coverage of the patch with intestinal neomucosa by 8 or 16 weeks [4, 12, 13]. While granulation tissue in the central area of the flap was detected in most of the 2 month rats, we observed that new intestinal mucosa completely covered the peritoneal side of the flap in all rats at 4 months.

In studies related to intestinal patching, the main goal is to achieve an adequately large flap or patch for growing neomucosa. An OPMP flap provided significant enlargement of the jejunal segment in the 2 and 8 month groups compared to the 4 month groups. In accordance with this finding, the contraction rate of the flap was highest in the 4 month group. At the present time we have no explanation for this finding, hence further investigations are warranted. Thompson et al. [4] reported that in a rabbit model the contraction rate of the colon serosa and the abdominal muscle was approximately 42%. The OPMP flap contracted by only 27.4% to 32.9% of the initial size. In this context, the silicon graft, which was used to create the OPMP flap, may be considered as an important factor preventing increase in the contraction rate.

Similarly to the macroscopic characteristics, microscopic evaluation of the flaps showed that neomucosal growth was poor in the 2 month group despite mature formation of the neomucosa in the 4 and 8 month groups. Well-developed villi covered the muscularis mucosa at the base of the lamina propria and smooth muscle in the 4 and 8 month rats. Two month rats had only a single layer of columnar epithelial cells on top of the fibrous tissue. This result indicated that a thin striated muscle layer may have transformed into fibrous tissue and that mature villi lamina propria and smooth muscle covered the fibrous tissue. In several experimental studies, it has been demonstrated that normal-appearing small bowel mucosa containing lamina propria, muscularis mucosa and smooth muscle was able to overlay serosal patches, abdominal wall pedicle flaps and prosthetic materials [3, 5, 12]. Villous height, crypt density and crypt depth of neomucosa, present at the lateral margin of the flap, were markedly low at 2 months. Bragg et al. [3] reported that villous length and crypt depth developed well at 8 weeks, although low functional neomucosa grew over intestinal defects patched with colonic serosa. Moreover, in a rabbit model the neomucosa formation over a flap of abdominal wall musculature had well-developed regular villi at 8 to 12 weeks. However no quantitative analysis of villous height, crypt density and crypt depth of the neomucosa was performed in this study [12].

With this flap, we attempted to design a new rat model for increasing intestinal surface area. The neomucosa growing on the musculo-peritoneal flaps based on omental leaves appeared macroscopically and microscopically to be quite similar to normal rat jejunum. An OPMP flap seems to produce adequate material for patching small intestinal remnants following massive bowel resection. Despite a two stage operation, the advantages of this technique are obvious. These include the possibility of using the abdominal muscle and peritoneum without jeopardising the bowel, the ability to achieve a large size for the musculo-peritoneal layer and a possible increase of absorptive capacity in the patched segment by OPMP flap [1]. Furthermore the utilisation of the OPMP flap in the repair of other organs such as the oesophagus and bladder appears to be worth considering.

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