Experimental Assessment of Small Intestinal Submucosa as a Small Bowel Graft in a Rat Model

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Background/Purpose: Small intestinal submucosa (SIS) is an extracellular matrix used in tissue engineering. The purpose of this study is to evaluate the feasibility of using SIS as a scafford for small bowel regeneration in a rat model.

Methods: A 2-cm length tubular SIS graft from donor Sprague Dawley rats was interposed with bilateral anastomosis in the median tract of an isolated ileal loop of Lewis rats used to construct an ileostomy. The grafts were harvested and analyzed at each of the time-points ranging from 2 weeks to 24 weeks after operation using histology and immunohistochemistry.

Results: Macroscopic examination found no adhesion in the surrounding area of neointestine by 24 weeks, and no stenosis was visible. The shrinkage of neointestine was indicated from 20% to 40%. Histologic and immunohistochemical evaluation showed that SIS grafts were colonized by numerous inflammation cells by 2 weeks. Neovascularization was evident, but the luminal surface was not epithelized. By 4 weeks, transitional mucosal epithelial layer began to line the luminal surface of the graft, and nearly 70% luminal surface of the graft had been covered by mucosal epithelium at 8 weeks. By 12 weeks, the luminal surface was covered completely by a mucosal layer with distinct bundles of smooth muscle cells in the neointestine. At 24 weeks, the neointestine wall showed 3 layers of mucosa, smooth muscle, and serosa.

Conclusions: The preliminary study suggested that SIS allow rapid regeneration of mucosa and smooth muscle and might be a viable material for the creation of neointestine.

INDEX WORDS: Small bowel, tissue engineering, small intestinal submucosa.

SHORT BOWEL syndrome is defined as the spectrum of malabsorption that occurs after resection of a major portion of the small intestine.[1-3] It is suggested that many patients with short bowel syndrome are dependent on total parenteral nutrition (TPN) and cannot be weaned off TPN because of the extensive resection or inadequate adaptation.[4] This type of nutrition support is associated with recurrent episodes of systemic infection and progressive cholestatic live disease. In addition, home-based TPN is accompanied by expensive costs estimated at approximately $100,000 per year.[5] Surgical treatment of short bowel syndrome at bowel lengthening or slowing intestinal transit to increase the absorptive surface area or time has been unsuccessful.[4,6,7] Small bowel transplantation as a promising treatment option has been promoted for patients with life-threatening complications from TPN. However, use of small bowel transplantation in man is still limited by unsolved immunologic problems[7,8] and organ donor shortage especially among the pediatric population.[9]

Small intestinal submucosa (SIS) is a membrane harvesting from the animal small intestine after which the tunica mucosa, serosa, and muscularis are removed, providing a collagen-rich membrane that is composed mainly of the submucosal layer.[10,11] SIS is unique from other previously used graft materials in that it contains functional growth factors that are likely vital to the regenerative process.[12] SIS has been shown to be biocompatible, resistant to infection, and induce tissue-specific regeneration in numerous tissues, including the blood vessels, the abdominal wall, urinary bladder, and tendons.[10,13-15] SIS also has undergone serial immunologic testing, and there has not been any evidence of rejection reaction.[16-18]

The investigation in tissue engineering for bioengineering small bowel has been initiated using biomaterials like SIS or polyglycolic acid by Vacanti and others.[19,20] The current study addresses the suitability of SIS grafts used as a scaffold for small bowel regeneration in the rat model.

MATERIALS AND METHODS

Animal Care

All animal care and use complied with the institutional regulations established and approved by the Animal Care and Use Committee of

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Kagawa Medical University for Animal Research Program. Sprague Dawley and Lewis rats were used in this study. Rats were kept in individual cages and fed standard rat chow and water ad libitum for a period of 1 week before use. Surgical procedures were performed under fluothane inhalational anesthesia.

Preparation of Small Intestinal Submucosa

Male Sprague Dawley rats were used as donors for the source of SIS. SIS was prepared as outlined previously in detail by Badylak et al.[13] In brief, segments of rat intestine were harvested and immediately placed in 0.9% saline solution. Segments then were cut into 2-cm lengths, and the mesenteric tissues were removed carefully from the segments. The segment of intestine then was everted (inside out), and the superficial mucosa was removed mechanically using a scalpel handle and moistened gauze. The segment then was everted again with the stratum compactum becoming the new luminal lining, as in its original orientation, and the serosal and muscularis layers were removed from its outer surface by the similar mechanical abrasion. This produces an extremely thin, translucent, whitish membrane tube (diameter, 4 to 6 mm) consisting of the submucosa with the attached stratum compactum and muscularis mucosa of the mucosa. The SIS then was rinsed with saline. To construct a multilayered tube, 4 layers of SIS were wrapped on a 6-mm diameter plastic tube and sewed at the edges of the SIS together with absorbable sutures. The SIS grafts then were sterilized in 75% ethanol, rinsed in saline solution again, and stored at 4°C in a 10% neomycin sulfate (Sigma, St Louis, MO) solution until use. Storage time for the SIS grafts ranged from 1 week to 3 months.

SIS Implantation

Adult male Lewis rats (n = 20) were used as recipients for this project. Under fluothane inhalational anesthesia, a midline abdominal incision was made, and the abdominal cavity was exposed. An ileal loop with several vascular vessels was isolated, whereas the interrupted bowel was reanastomosed to maintain the normal alimentary canal. A 2-cm in length tubular SIS graft was interposed in the median tract of the ileal loop and anastomosed in an end-to-end fashion using interrupted 7-0 absorbable polydioxanone sutures (Fig 1). Two to 4 silk sutures were added to the anastomosis to act as a marker for later identification. Before completion of anastomosis, a silicon stent was placed in the loop. The ileal loop then was used to construct a double ileostomy at each side of the abdominal incision (Fig 2). The abdominal incision was closed in 2 layers with 4-0 nylons.

Postoperatively, animals were maintained on a liquid diet and water for 48 hours and then recommenced on a full diet of rat chow. Antibiotics were used for 1 week. The loop stent was kept in place for 12 weeks and then removed. Loop washes were performed with saline solution through the ileostomy one time every other day for more than 12 weeks.

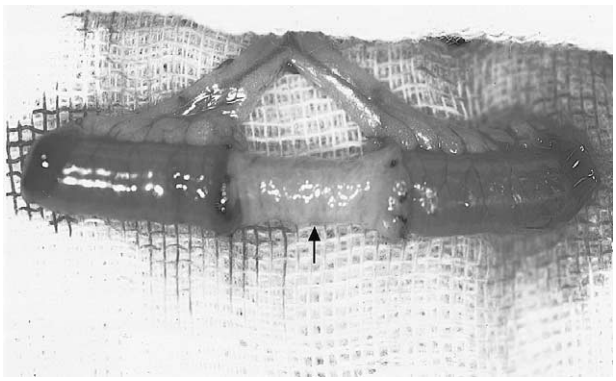

Fig 1. Isolated ileal loop with interposed SIS graft before double ileostomy.

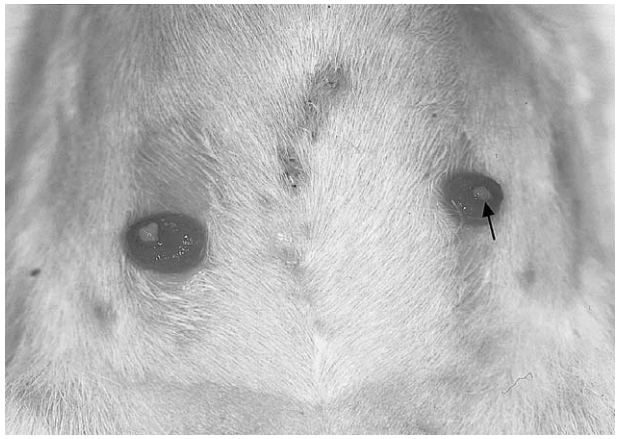

Fig 2. A double ileostomy at either side of the abdominal midline at 8 weeks. Some luminal contents were excreted from the stoma (arrowhead).

Specimen Assessment

Scheduled euthanization was carried out at various time points ranging from 2 to 24 weeks. The specimens were harvested, and the silk suture area along with luminal size was measured. The specimens then were submitted for histologic, histochemical, and immunohistochemical assessment. Specimens were fixed in formalin, dehydrated, and embedded in paraffin wax. Sections were cut at 4 μ m and stained with H&E and Masson's trichrome stains. The presence of smooth muscle cells and innervation of remodeling graft were checked by immunohischemical staining using monoclonal mouse antihuman α-smooth muscle actin antibody (Dako Corp, Carpinteria, CA) and antiS100 (Sigma, St Louis, MO). Sections were dewaxed and rehydrated. After the endogenous peroxidase activity blocking, sections were incubated with a primary antibody diluted 1:1000 for 10 minutes with the LSAB2 systems (Dako Corp, Carpinteria, CA). Sections then were washed, and the antibody binding sites were revealed by incubating the sections in a solution of diaminobenzidine hydrochloride (DAB) (Dako Corp, Carpinteria, CA). The normal intestinal smooth muscle was stained as a positive control.

RESULTS

Of the 20 animals receiving the SIS grafts implantations, 2 rats were killed within 2 weeks owing to the operative complications related to stenosis or peritonitis secondary to the leakage at the anastomotic site. The other 18 rats survived up to the time of planned harvest. Macroscopically, there were adhesions between the SIS graft and the surrounding tissue, and the lumen of the graft was filled with some mucous materials at 2 weeks. By 24 weeks, the regenerated bowel had no adhesion to the surrounding tissue, but some fibrous scar tissue surrounding its external surface was present in all animals. There was no evidence of obstruction or stenosis at the anastomotic site, and the lumen was patent with some mucous materials. Diameter of the regenerated bowel

Table 1. Survival Time and Shrinkage Degree of Regenerated Bowel

Rats (No.)	Survival time (wk)	Degree of shrinkage (%)
3	2	18.5 ± 3.2
3	4	22.1 ± 5.9
4	8	25.6 ± 7.4
4	12	35.5 ± 6.6
4	24	38.4 ± 8.5

dilated slightly compared with the proximal and distal native small bowel. However, based on the silk suture marker, the shrinkage of the regenerated bowel at the length was identified compared with the original length of SIS graft, indicating a 20% to 40% contraction (Table 1). The changes in the shrinkage degree appeared to be evident after 12 weeks.

Histologically, at 2 weeks a moderately intense inflammatory response was observed in the SIS graft, and fibroblasts and mononuclear cells infiltrated the collagen fiber of the graft. Neovascularization appeared and could be seen in the wall of the graft, but the luminal surface was not covered with epithelium (Fig 3A). At 4 weeks, transitional mucosal epithelial layer began to line the luminal surface of the graft at both anastomotic ends, and the graft was beginning to degrade. By 8 weeks, nearly 70% luminal surface of the graft had been covered by mucosal epithelium (Fig 3B). In the central region of the graft without the epithelial lining, mononuclear inflammatory cell infiltrations were minimal, and fibroblasts and small capillaries were predominant. There were scattered smooth muscle cells with morphologic and staining features consistent with smooth muscle cells of native small bowel (Fig 4). At 12 weeks, the luminal surface of the graft was covered completely by a relatively well-developed epithelial layer with numerous villi (Fig 3C). The submucosa layer was not evident. Immunohistochemical staining showed a smooth muscle layer was present with distinct bundles of well-formed smooth muscle cells in the regenerated bowel, especially near both anastomotic sites (Fig 5B). There were no signs of the inflammatory reaction. Finally, by 24 weeks, the regenerated bowel wall showed a well-developed 3 layers of mucosa and smooth muscle and serosa and was similar to the normal bowel (Fig 3D). However, the quantity and organization of smooth muscle fibers differed slightly from that seen in the normal small bowel (Fig 5A,C). Circular muscle layer appeared predominant, and longitudinal muscle layer was not evident. The submucosa appeared more evident than before. Immunohistochemical staining showed no innervation of regenerated bowel in 24-week samples.

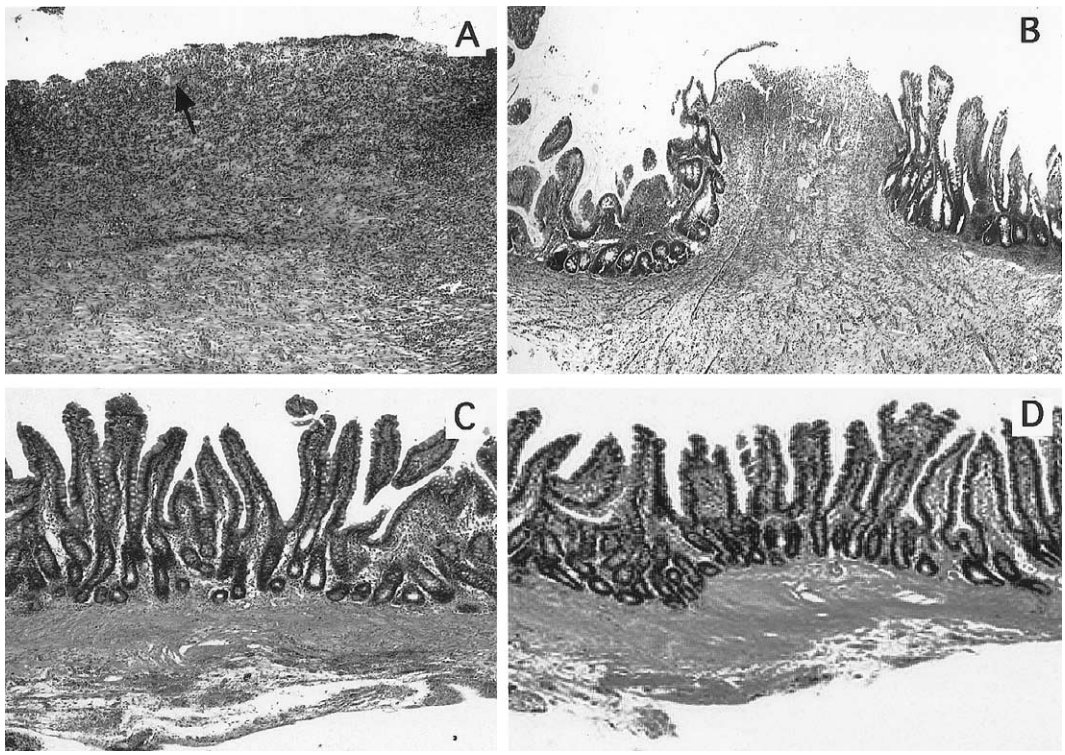

Fig 3. H&E-stained histologic photomicrographs of SIS-regenerated rat small bowel tissue. (A) No epithelization at the luminal surface by 2 weeks. Arrows depict neovascularization. (B) Major portion of the epithelized graft luminal surface by 8 weeks. Inflammatory reaction was minimal. (C) A well-organized mucosal epithelial layer with smooth muscle cells regeneration by 3 months. (D) A well-developed 3 layers of mucosa, smooth muscle, and serosa of the regenerated bowel wall (original magnification ×100).

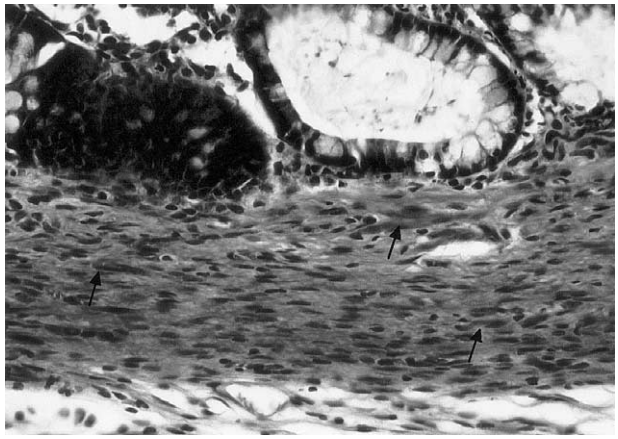

Fig 4. Masson's trichrome-stained histologic photomicrographs of SIS-regenerated rat small bowel tissue by 8 weeks. Arrows indicate regenerated smooth muscle cells scattered within the SIS graft (original magnification ×250).

DISCUSSION

Recently, SIS, a new biodegradable collagen-rich, nonimmunogenic biomaterial has been shown to induce tissue remodeling in animal models of large and small arterial grafts,[13,21] large diameter venous grafts,[22] urinary bladder repair,[10] ligament and tendon repair,[15] and treatment for body wall defects.[23] In these models, SIS was found to be biocompatible, resistant to infection, able to induce rapid neovascularization, and able to remodel into site-specific tissue with histologic structures resembling the native tissue. In our study, we attempted to use rat SIS as a scaffold for the bowel regeneration. Rat SIS was created easily and successfully from the donor small bowel. Although the resulting translucent tube was extremely thin, it could be easily fashioned into 4-layer tube with its integrity and held sutures firmly.

To determine if SIS grafts could be used as a scaffold for the small bowel regeneration, an isolated ileal loop with an ileostomy was created, and tubular SIS graft was interposed in the median tract of the loop. The loop with ileostomy could protect it from the alimentary transit, which may induce the bare area without epithelization in the graft luminal surface, because the mixture of luminal contents and necrotic tissues in the alimentary tract could attach to the graft luminal surface and could not be drained out by the regenerated small bowel with no motility. Moreover, retained contents may lead to inflammatory reaction on the graft to disturb the regeneration. Therefore, we washed the loop frequently to avoid this. The SIS graft did allow epithelial regeneration within its lumen and new blood vessel ingrowth. This remodeling process occurred as early as 2 weeks primarily from mononuclear and fibroblastic cell invasion with new blood vessel formation, and subsequently the SIS graft began to be lined with epithelial layer. By 24 weeks, the neointestine showed a well-organized 3 layers of mucosa, smooth muscle, and serosa similar to normal small bowel. The lumen was patent, and no stenosis was observed, although there was some graft shrinkage. The results suggested that SIS allowed not only rapid mucosal epithelium regeneration and ingrowth of new blood vessels but also smooth muscle fibers to have a regenerative capacity. We found that smooth muscle regeneration developed from the graft edges by 8 weeks, and morphologically circular smooth muscle cells appeared predominately. This finding shows a possibility of the ingrowth of native muscle from the graft edges consistent with other reports.[10,24] Sandusky et al[16] suggested another possible explanation for smooth muscle regeneration caused by muscle production derived from pericytes accompanying the capillary endothelial cells.

Our histologic findings indicated that host remodeling of the SIS graft was characteristic of a fibrovascular healing-type reaction.[25] It developed initially from mononuclear and fibroblastic cell infiltration with neovascularization, which clearly was visible after 2 weeks and present in the whole wall of SIS graft. Then the SIS graft began to be covered with mucosal epithelial layer

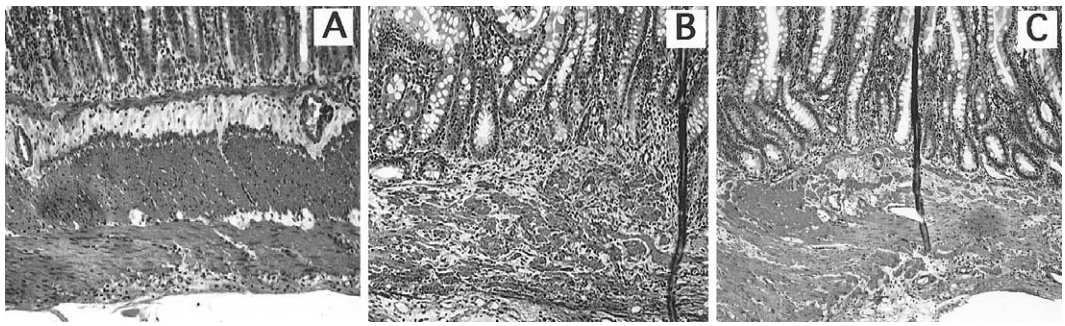

Fig 5. Immunohistochemically stained normal small bowel and SIS-regenerated rat small bowel tissue. (A) Normal small bowel. (B) SIS-regenerated rat small bowel by 3 month indicating bundles of well-formed smooth muscle fibers in the regenerated bowel. (C) SIS-regenerated rat small bowel by 6 months show some difference in organization of the smooth muscle layer than (A) (original magnification ×250).

and mononuclear inflammatory cell infiltration decreased to be minimal. There was no histologic evidence of foreign body rejection during the whole experimental period. The mechanism of SIS nonimmunogenic feature is not clear. Allman et al suggested that implanted SIS graft elicits a vigorous immune response, but this response is restricted to the TH2 pathway, which may allow acceptance and remodeling of the graft tissue.[26]

Recently, various biodegradable materials have been used in intestinal tissue reconstruction. Thompson et al[27] attempted to use prosthetic materials and an absorbable polyglycolic acid for engineering neointestine, and the results were unsuccessful. Choi and Vacanti[19] and Kaihara et al[28] used polyglycolic acid (PGA) as a scaffold for the formation of cyst structures created by seeding intestinal organoids harvested from 7-day-old rat into PGA. The result was enterocytic cysts with neomucosa grown on the polymer but with no sign of smooth muscle regeneration. Chen and Badylak[20] evaluated the use of SIS for bioengineering neointestine in a dog model. They found that SIS patch showed the 3 layers of mucosa, smooth muscle, and serosal covering, but failed to make a tubular segmental replacement of the small bowel with SIS.

There is concern of potential infection regarding the use of any foreign material for tissue replacement. Synthetic grafts tend to become infected more easily than biomaterial grafts. Because biomaterials can stimulate revascularization, they provide a microenvironment that discourages bacterial growth. Sarikaya et al[29] indicated that extracellular matrices derived from SIS possess antimicrobial activity. The pathologic findings in our study showed one feature of SIS graft involving early capillary growth into the graft tissue. The rich and rapidly capillary blood supply to this graft is probably responsible for graft viability and infection resistance.[17]

The most challenging aspect of small bowel tissue engineering is to require the recovery of peristaltic activity of the regenerated bowel. This recovery needs both smooth muscle development and reinnervation of the regenerated bowel. We identified the smooth muscle layer regeneration, but reinnervation was not shown immunohistochemically in our experiment using a 24-week specimen sample. Reinnervation may take much more time to develop. In addition, regenerated smooth muscle is in an immature state, as evidenced by the histology, and innervation may develop with regenerated smooth muscle maturation.

This initial study showed that SIS allowed rapid ingrowth of new blood vessels and epithelial and smooth muscle regeneration. SIS-regenerated rat bowel contained all 3 layers of small bowel. SIS appears to have the unique capability of acting as a biodegradable scaffold, promoting the regeneration of host tissue. Therefore, the use of SIS for small bowel tissue engineering could be a safer and viable alternative in the therapy of small bowel syndrome. Long-term studies are needed to focus on the functional aspect to determine whether regenerated smooth muscle and innervation eventually develop and function physiologically well.

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