

## Small Bowel Tissue Engineering Using Small Intestinal Submucosa as a Scaffold

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**Background.** Small intestinal submucosa (SIS) is an extracellular matrix used in tissue engineering studies to create *de novo* abdominal wall, urinary bladder, tendons, blood vessels, and dura mater. The purpose of this study is to evaluate the feasibility of using SIS as a scaffold for small bowel regeneration in an *in situ* xenograft model.

**Materials and methods.** Twenty-three dogs had a partial defect created on the small bowel wall which was repaired with a SIS patch. Four dogs underwent small bowel resection with placement of an interposed tube of SIS. The animals were followed 2 weeks to 1 year.

**Results.** Three of the 23 dogs with SIS placed as a patch died shortly after surgery due to leakage from the site. The other 20 dogs survived up to time of elective necropsy with no evidence of intestinal dysfunction. At necropsy, the bowel circumference in the patched area had no stenosis. Histological evaluation showed the presence of a mucosal epithelial layer, varying amount of smooth muscle, sheets of collagen, and a serosal covering. Architecturally, the layers were not well organized in the submucosal region. An abundance of inflammatory cells was present in the early postoperative period but receded with time. All 4 dogs with a tubular segment of SIS interposed had significant problems. One had partial obstruction at 1 month, and 3 died in the early postoperative period due to leakage.

**Conclusions.** This preliminary study suggests that SIS patches can be used for small bowel regeneration. Tubular segmental replacement is not feasible at this time. © 2001 Academic Press

**Key Words:** tissue engineering; bowel; extracellular

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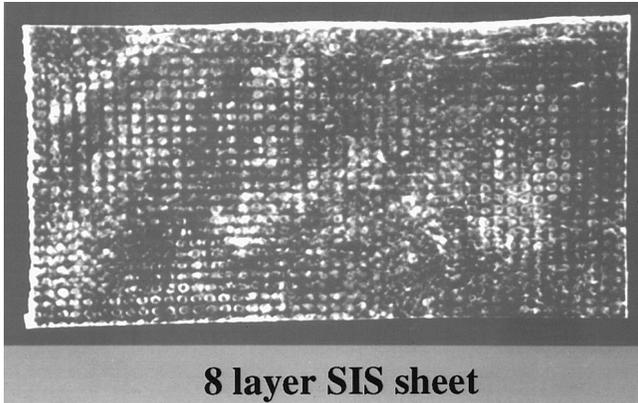
**matrix; small intestinal submucosa; short gut syndrome.**

### INTRODUCTION

In 1966, Matsumoto described the use of inverted small intestine to replace large veins in dogs [1]. Improved processing of this biomaterial (porcine small intestinal submucosa, SIS) has made it readily available for tissue engineering studies. SIS is a relatively acellular collagen-based matrix from which extensive *in situ* tissue remodeling has been demonstrated in both rat and canine models. It has been used as a scaffold for regeneration of a variety of tissues including the abdominal wall, urinary bladder, tendons, blood vessels, and dura mater [2–7]. Grossly and microscopically, the remodeled tissue resembles the native tissue. We previously demonstrated that it may be used as a patch for esophageal healing [8], but its use for remodeling and regenerating small bowel has not been previously investigated.

Small intestinal replacement remains a complex problem with most of the needs arising from patients with short bowel syndrome. These patients often have lost >75% of their small bowel and suffer from malabsorption, malnutrition, electrolyte imbalance, diarrhea, and vitamin deficiency. The principal causes of short bowel syndrome in children and infants are gastroschisis, intestinal atresias, necrotizing enterocolitis, and volvulus. In adults, the most common causes are vascular accidents, Crohn's disease, and tumors.

The current therapy for short bowel syndrome is limited. Total parenteral nutrition can be used to provide supportive care but is obviously not curative. It is fraught with long-term complications including liver failure, intravenous access limitations, and recurrent infections secondary to the presence of a foreign body.



**FIG. 1.** Processed 8-layer sheet of SIS. This material is soaked in saline to make it pliable prior to use.

Additionally, home-based parenteral nutrition is expensive with costs estimated at approximately \$50,000/year.[9] Strategies in surgery are limited to increasing absorptive surface area and slowing the transit time, neither of which is satisfying or successful [10–14]. Finally, small bowel transplantation is still a relatively novel therapy and remains a highly morbid procedure. Recent data from Pittsburgh showed 1 year patient survival of 70% and 5 year survival of 50% [15–16]. Graft survival at 1 and 5 years was 60 and 40%, respectively. Over 90% of patients experience at least 1 rejection episodes and long-term immunosuppression results in significant problems. These include recurrent infections and secondary malignancies. Finally, Vacanti and others have initiated investigations in tissue engineering and in the use of other biomaterials as a way of providing novel small intestinal tissue.[17–22] This field is still in its infancy, but early investigative studies appear promising. The purpose of this study was to evaluate the feasibility of using SIS as a scaffold for small bowel regeneration in an *in situ* xenograft model.

## MATERIALS AND METHODS

### *Small Intestinal Submucosa Preparation*

Badylak and others have described complete description of SIS preparation [2]. A brief summary is provided here. Freshly harvested porcine jejunum is obtained from a local slaughterhouse. It is inverted and the superficial layers of the tunica mucosa are removed by scraping with a knife handle. The tissue is then reverted to its original orientation and the serosa and tunica muscularis are removed. The resulting membrane is approximately 80–100  $\mu\text{m}$  thick and consists of the tunica submucosa and the basilar portion of the tunica mucosa. The sheets of SIS are sterilized by exposure to 0.1% peracetic acid. To make multilayered SIS, the appropriate number of SIS sheets are compressed and allowed to dry (Fig. 1). They are then gas-sterilized and kept until ready to use. Resoaking in saline prior to use makes the SIS sheets pliable and soft. Tubular SIS segments were made by wrapping a sheet of SIS over an appropriately sized glass tube and sewing the edges of the SIS together with an absorbable suture.

### *Animal Studies*

All animal studies were done after obtaining the appropriate approval from the institutional review board. All dogs were female under 2 years old with an approximate weight of 20 kg. Each dog was fasted overnight and given a preoperative dose of intravenous cefazolin. They were given sodium pentobarbital and maintained on inhaled isoflurane during the procedure.

Twenty-three dogs were randomly chosen for placement of a SIS patch in the small intestine. Because of the preliminary nature of this study, both a single layer and a multilayered configuration of SIS were used to identify the ideal type of material for the study. Laparotomy was done and a portion of small bowel wall was resected and patched with a single layer of SIS in 8 dogs and with a multilayered SIS in 15 dogs (Fig. 2). Care was taken to ensure that the luminal (i.e., mucosal side of the SIS) was kept inward on the luminal side. The SIS patch was sutured to the bowel using running 4-O Vicryl sutures and reinforced with interrupted 4-O silk sutures. The silk also acted as a marker for later identification of the patch site. In addition, all grafts were covered loosely with the omentum. The omental patch was utilized to minimize and contain a potential leak. A dog's omentum is very thin and would not be useful for patching the substantial defect we created in the small bowel for this study. The size of the patch averaged  $7 \times 3$  cm and approximated the size of the defect created on the small bowel. After completing the patch, the site was tested for leaks by compressing intraluminal fluids against the suture line.

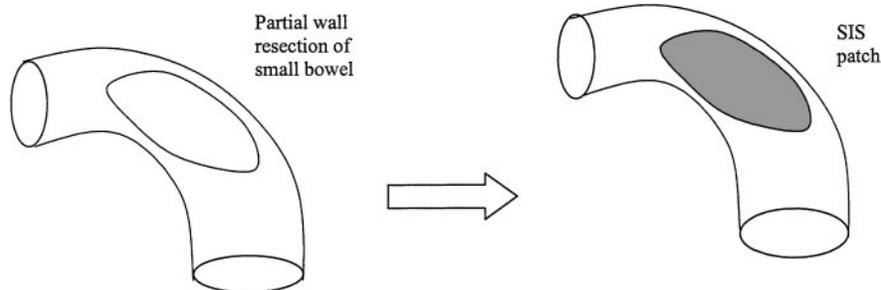
Four dogs had a small segment of their small bowel resected and reanastomosed with a tubular segment of SIS interposed (Fig. 3). The SIS tube was 6 cm long with a diameter of 2 cm. All the anastomoses were carried out using the same two-layer technique (4-O Vicryl and 4-O silk).

Postoperatively, food was withheld for 1 week and the animals were maintained on intravenous fluids and given antibiotics. The animal's weight and oral intake were closely monitored. Planned necropsy was carried out at various time intervals ranging from 2 weeks to 1 year. Intravenous injection of sodium pentobarbital (90 mg/kg) was used for the euthanization. However, if any of the animals appeared ill or septic, they were euthanized. If there was a problem with feeding and the dog appeared to have bowel obstruction, they were also euthanized. The operative site was harvested and the silk suture area was measured. The luminal size was also measured and the harvested bowel was prepared for hematoxylin and eosin staining.

## RESULTS

The results of the single and multilayered SIS were not significantly different. Thirteen out of 15 dogs that had a multilayered SIS placed as a patch survived and were healthy up to the time of elective necropsy. They tolerated their oral intake without any evidence of intestinal dysfunction and maintained their weight (initial wt  $24 \pm 2$  lb vs  $25 \pm 2.7$  lb). Two dogs were sacrificed within the first postoperative week because they appeared ill with fever, drooling, and lethargy. Peritonitis secondary to leakage from the patch site was found in both dogs.

Of the eight dogs that had a single layer of SIS placed, seven animals tolerated oral intake and survived up to the time of planned necropsy without any problems. One dog was euthanized on the second day after surgery due to illness and leakage from the surgical site was identified.



**FIG. 2.** Partial wall resection of the small bowel with subsequent repair of the defect with a SIS patch.

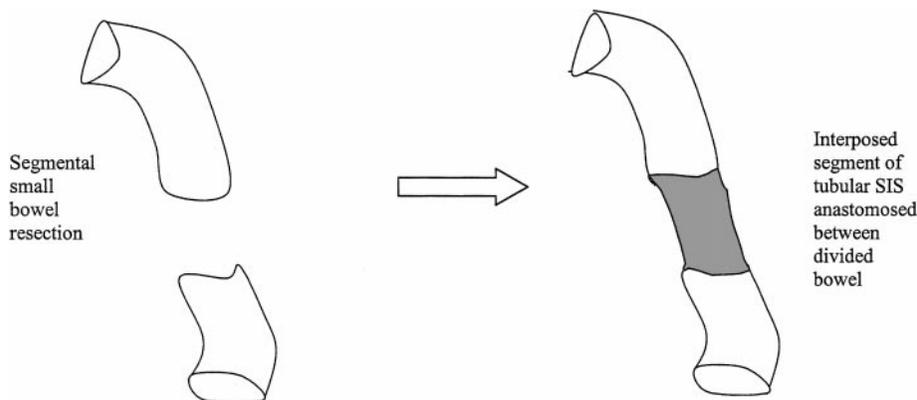
All four dogs that had a tubular segment of SIS interposed between the divided small bowel had significant morbidity. One dog had difficulty eating after 1 month with subsequent weight loss and lethargy. A partial obstruction secondary to luminal stenosis was found at necropsy. The other three dogs became ill early in the postoperative period and were found to have leaked from the anastomotic site.

At elective necropsy, the intestinal lumen at the site of the SIS patch was of normal size when compared to the surrounding small bowel. The gross specimen appeared similar to the native bowel both visually and texturally (Fig. 4). There was no evidence of any obstruction with no proximal small bowel dilatation in any of the dogs. The diameter of the regenerated bowel was exactly the same as the bowel proximal and distal to this patched area. On animals that were evaluated after 6 months, the patched area would have been difficult to identify by simple observation or palpation without the silk suture that was used to mark the patch because the regenerated bowel appeared identical to native bowel in size, texture, and consistency. The adhesions in the surrounding area made the identification easier, but to find the exact patched area, one could not discern the specific area with gross observation alone. The area of the SIS patch was smaller than when measured at the time of placement. The further out from the time of surgery, the smaller the residual

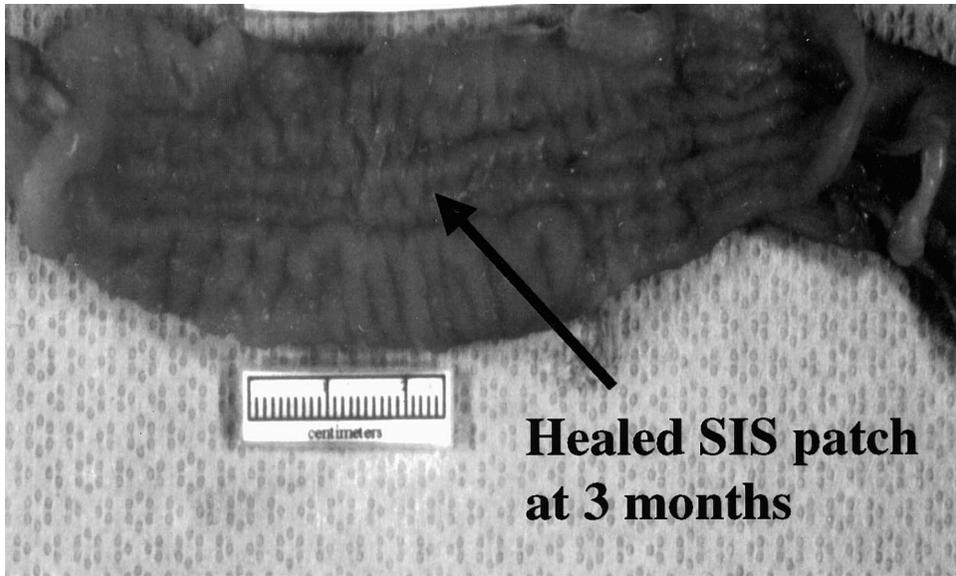
size of the area marked by the silk sutures. There was an approximately 35% decrease in the size of the patched area at 2 weeks. This area contracted to 50% of the initial area at 4 months and achieved maximal contraction of 80% at 1 year. Grossly, minimal inflammation and adhesions were noted around this patched area. Architecturally, at 2 months, the layers were not well organized in the submucosal region of the remodeled tissue. The best organized layer was the mucosa (Fig. 5). There was an abundance of inflammatory cells present in all configurations of SIS, but the amount of inflammation receded with time. Histologic studies revealed complete absence of the SIS by 3 months. At 6 months and beyond, histologic evaluation of harvested specimens showed that the layers of the remodeled wall contained a mucosal epithelial layer, varying amount of smooth muscle, sheets of collagen, and a serosal covering and appeared nearly identical to the native bowel (Fig. 6).

## DISCUSSION

The current support and treatment for short bowel syndrome are limited and dissatisfying. Intravenous nutritional support through the use of hyperalimentation can be successfully accomplished but is fraught with problems. Mortality in the pediatric population remains at 20% and is markedly worse in infants [23].



**FIG. 3.** Segmental small bowel resection and repair by interposing a segment of tubular SIS between the divided bowel.

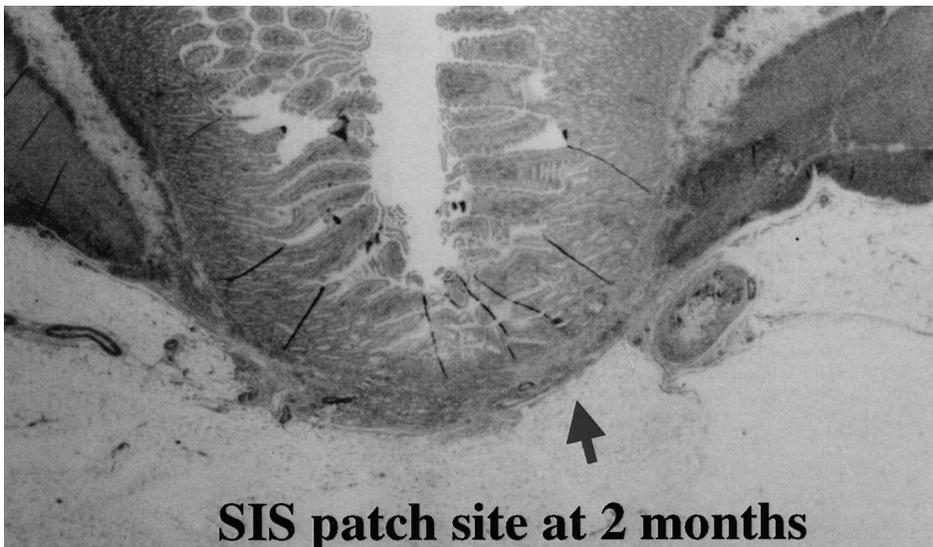


**FIG. 4.** Gross specimen of regenerated and healed small bowel at 3 months. There is no difference in the texture or size of the lumen at the patched area compared to the native bowel.

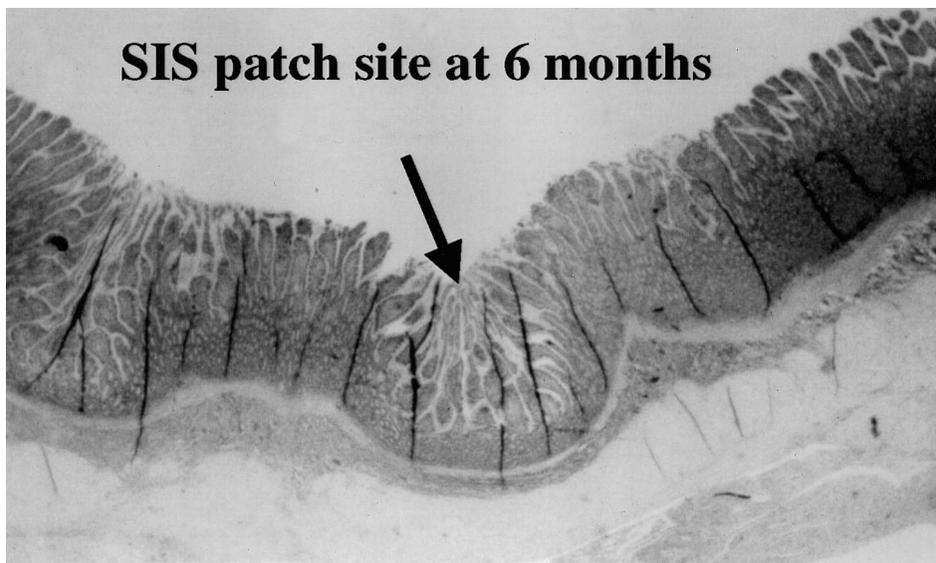
Hepatic failure accounts for approximately 50% of these deaths. Surgical alternatives such as bowel lengthening and valves are only partially successful. Intestinal transplantation still is in a relatively early stage and the need for long-term immunosuppression is daunting. Tissue engineering using both *in vivo* and *in vitro* models appears promising. Creation of neointestine through tissue culture techniques or through the use of applicable scaffolding can offer significant advantages over the current modes of therapy.

Small intestinal submucosa has been placed in a variety of sites resulting in the regeneration of tissues that resemble the native tissues both grossly and mi-

croscopically. Furthermore, functional studies suggest that the regenerated tissues have characteristics similar to the native tissues [24–30]. Aside from our previous report in its use in the esophagus [8], this is the first report of the use of SIS as a scaffold for the creation of neointestine. The use of SIS as a framework for the generation of novel intestinal tissue makes intuitive sense since the originating biomaterial comes from a gastrointestinal source. Our study suggests that SIS can be sewn to a piece of bowel and new bowel surface will grow over this sheet of collagen matrix. The collagen matrix is resorbed in the first 2–3 months and a multilayered tissue that resembles the native



**FIG. 5.** Histologic result observed at 2 months with the presence of immature mucosa and submucosa. Additionally, there is a relative absence of smooth muscle across the patched area.



**FIG. 6.** At 6 months, the histologic results demonstrate the presence of all layers—mucosa, submucosa, smooth muscle, and serosa. There is minimal architectural difference between the native and the regenerated bowel.

small bowel is produced. By 3 months, no evidence of the patched SIS was found on histologic evaluations. This correlates with other investigators who have used this material for bladder regeneration [28–30].

Thompson and his colleagues previously demonstrated that utilizing the cecum's serosa as a patch to cover an intestinal defect results in formation of neomucosa [31]. They subsequently showed that the utilization of prosthetic materials (Dacron and polytetrafluoroethylene) and an absorbable biomaterial (polyglycolic acid) were not useful in creating neointestine [32]. A criticism of our model may be that SIS is no better than a simple serosal patch in providing a base for intestinal regeneration. Our study is a preliminary evaluation of the potential use of a biomaterial that may serve as a scaffold for bowel growth. We created a defect not only through incising into the small bowel as done by others such as Thompson, but we also removed 50% of the circumference of the bowel wall where this SIS patch was placed. The *in situ* model used in this study verifies that SIS can serve as a scaffold for the complete regeneration of all layers of the intestine. Not only was neointestine regenerated over the scaffold, but no narrowing of the bowel occurred despite having removed 50% of the bowel circumference prior to patching. Although we did not officially “increase” the intestinal absorptive surface, by maintaining the circumference of the patched bowel, SIS did add to the surface area. SIS may be eventually be identified as one alternative for increasing the absorptive surface of patients with short bowel syndrome if a sufficiently large piece of biomaterial can be engineered to promote intestinal growth. However, many more studies will be needed to achieve that success.

We used a variety of configurations of SIS material

in this study because we had no preconception of which would work best. The initial use of a single layer material was technically harder. The single layer of SIS was too flimsy and difficult to handle. Subjectively, the multilayered SIS was technically easier to manipulate and it held sutures without tearing.

In another exploratory fashion, we created a tubular piece of SIS by sewing the edges of the SIS sheet together over a glass rod of the approximate diameter of the dog's intestine. We wanted to see if the simple placement of a circumferential piece of SIS between divided bowel would result in healing and bowel regeneration. This configuration failed in our hands. Three out of four dogs became septic after leakage from the anastomotic site. The one survivor developed bowel obstruction at 1 month due to a significant stenosis. The tube probably collapsed upon itself and became stenotic in the one dog that did not leak. The failure of this technique may be secondary to the length of the tube and the need for a closer contact with the native tissue and the associated “nutrients.” This may indicate that there is a limitation to the size of the SIS that could be expected to become covered with intestinal tissue after patching.

Histologically, the SIS patch showed the presence of mucosa, submucosa, smooth muscle, and serosal covering. The architectural arrangement suggested a healing process with an initial abundance of inflammatory cells that receded with time. An argument could be made that the regenerated bowel was simply healed normal bowel that would have occurred without the presence of a framework such as SIS. If 50% of the small bowel wall is removed as may occur with intestinal injuries, there is usually stricture formation after healing [33–36]. The surgical tenets call for a bowel

resection in these situations. All of the healed SIS patches had remodeled bowel present without any luminal narrowing. Other evidence that points to regeneration of the small bowel over the SIS scaffold is the presence of acute inflammatory process and early remodeling that occurs in the dogs that underwent necropsy in the first 2–4 weeks of surgery. This inflammatory process is the appropriate response generated by the host tissue after the trauma of bowel resection and the addition of a foreign object to the healing field. The presence of smooth muscle in the regenerated bowel is reminiscent of other studies involving SIS [28–30]. In particular, bladder augmentation procedures using SIS have resulted in the formation of bundles of smooth muscle cells in the regenerated tissue. There may be some inherent property in this biomaterial that is conducive to smooth muscle formation. Other studies will need to be conducted to clarify this tissue.

Vacanti and his colleagues have also been investigating ways to bioengineer intestine [17–18]. They have focused on using a different biomaterial termed polyglycolic acid (PGA). They have seeded this biomaterial with intestinal organoids harvested from neonatal rats prior to implanting the construct onto an adult rat's omentum. This resulted in the formation of a cystic structure that resembled enteric cysts. They have not attempted to use PGA alone as a scaffold for intestinal regeneration in an *in situ* model.

In summary, small bowel replacement remains an enigma and tissue engineering may prove to be a safer and more satisfying alternative to the current therapeutic armamentarium. SIS patches can be used to increase the absorptive surface as we have demonstrated in our xenograft model. However, tubular segmental replacement of the small bowel with SIS is not feasible at this time. This is a very preliminary evaluation of the use of SIS in the creation of neointestine. Future studies are needed to focus on the functional aspect of the regenerated small bowel in addition to finding ways to improve the cellular histology and architectural organization that occurs during the remodeling process.

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