Evaluation of Porcine-Derived Small Intestine Submucosa as a Biodegradable Graft for Gastrointestinal Healing

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High-risk anastomoses in the gut may benefit from the application of a synthetic reinforcement to prevent an enteric leak. Recently a porcine-derived small intestine submucosa (SIS) was tested as a bioscaffold in a number of organ systems. The aim of this study was to evaluate the effectiveness of SIS in stimulating healing in the stomach. Twelve rats underwent surgical removal of a full-thickness gastric defect (1 cm) and subsequent repair with a double-layer patch of porcine-derived SIS. The graft was secured with interrupted sutures placed within 1 mm of the edge of the graft. After 21 days, the animals were killed and their stomachs harvested for histologic examination. Cross sections were processed for paraffin embedding and 4-micron sections were stained with hematoxylin and eosin. All animals survived, gained weight, and demonstrated no signs of peritonitis over the 3-week postoperative period. On postmortem examination, the defect was completely closed in all animals by granulation tissue and early fibrosis. Although most of the luminal surface of the grafted areas remained ulcerated, early regeneration of normal gastric mucosa was seen at the periphery of the defect. SIS may act as an effective scaffolding agent for intestinal mucosa and may offer protection in high-risk anastomoses. (J GASTROINTEST SURG 2003;7:96–101.)

KEY WORDS: Small intestine submucosa, porcine, SIS, anastomosis, perforation

Gastrointestinal anastomosis failure represents a devastating postoperative complication that increases the hospital stay and the perioperative mortality by two- and threefold, respectively.[1] Despite improved perioperative care and the advent of new operative techniques, anastomosis leakage continues to constitute a significant health problem. Several risk factors have been associated with anastomosis breakdown including the use of corticosteroids, bowel obstruction, low serum albumin levels, and the need for intraoperative blood transfusions.[2] Commonly recommended precautions to enhance complication-free healing of the anastomosis include a sufficient blood supply to the ends being anastomosed, minimal tension between edges, adequate preoperative preparation, and proper surgical technique. Because of the multifactorial nature of and difficulty in preventing anastomotic dehiscence, a variety of products have been tested to provide protection to the anastomosis site.[3–6]

Recently, porcine-derived small intestine submucosa (SIS) has been introduced as a reinforcement of soft tissue.[7] SIS is an acellular collagen-based matrix primarily composed of fibrillar collagens (types I, II, and V)[8] that enhance healing while stimulating a minimal immune response.[9] Studies have shown that SIS serves as a bioscaffold for generation of a wide variety of tissues and organs such as urinary bladder,[10,11] dura mater,[12] blood vessels,[13–15] and tendons.[16] In the present study we evaluated the effectiveness and regenerative properties of SIS in a rat model of gastric perforation. We show that in vivo implantation of SIS provides contingency and facilitates ingrowing of native gastric tissue into the matrix.

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MATERIAL AND METHODS

Surgical Manipulation and Study Design

Twelve adult Wistar rats (Charles Rivers Laboratories, Raleigh, NC) weighing 300 to 350 g were housed in cages with free access to water and food under standard laboratory conditions (room temperature 23° C; 12 hr dark-light cycles). Rats were weighed before surgery and at regular intervals after operation. Food was withheld but not water for 24 hours before surgery. On the day of surgery, anesthesia was induced and maintained with isoflurane and oxygen, and the abdominal area was prepared in the usual manner. A 3 cm midline laparotomy was performed, and the stomach was identified and gently mobilized with atraumatic forceps. A 1 cm full-thickness defect was created in the body of the stomach, which compromised approximately 40% of the area of the anterior gastric wall. After hemostasis was achieved, a two-layer SIS patch (Cook Biotech, West Lafayette, IN) was prepared and secured to the gastric wall with interrupted 5-0 Prolene sutures. Stitches were taken from the seromuscular layer and placed within 1 mm of the edge of the graft. In a subset of animals (n = 6), the operation was completed with an omentectomy. The incision was closed in two layers, and the animals were allowed to recover from anesthesia. Postoperatively animals were checked on a daily basis for signs of distress, and pain medication was administered as needed (morphine, 2mg/kg). Body weight, temperature, and food intake were monitored daily. All aspects of this research were approved by the Durham VA Medical Center Animal Care and Use Committee (Durham, NC).

Small Intestine Submucosa

The material was supplied sterile in a peel-open package and rehydrated for 10 minutes in saline solution before implantation. Soaking the material makes the sheets pliable and soft. For the present experiments, a double patch, 1.2 cm in diameter, was created from the SIS sheet by opposing two layers. Special care was taken to ensure that the luminal sides of the material were kept inward.

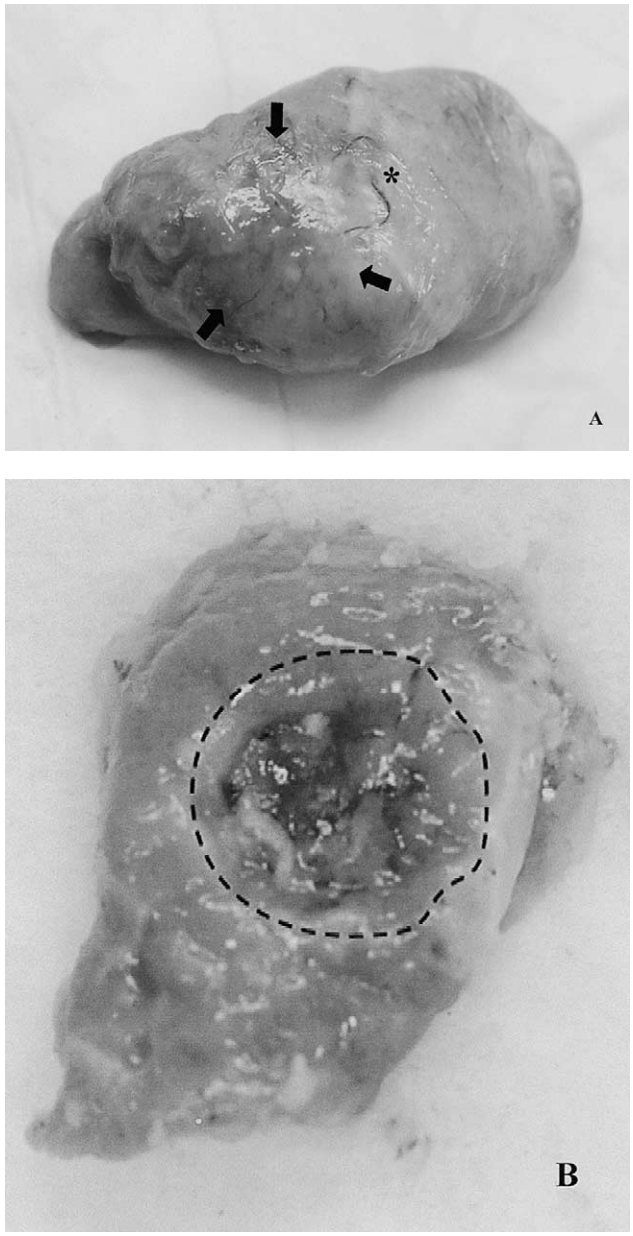

Fig. 1. Macroscopic view of external and luminal gastric wall 3 weeks after implantation. **A,** Serosal surface of gastric wall. The material was completely incorporated into the gastric wall making it difficult to distinguish between graft and normal tissue. *Arrows* denote grafted area; *asterisk* shows suture material. **B,** Luminal side of the stomach. *Broken line* denotes former defect.

Histologic Evaluation

After 21 days, the animals were killed and their abdominal cavities were macroscopically evaluated for adhesions. The grafted area surrounded by intact gastric tissue was excised and fixed in 10% neutral buffered formalin. Cross sections of the grafted area were processed for paraffin embedding, and 4-micron sections were stained with hematoxylin and eosin. All specimens were reviewed by one of us (M.G.), who was unaware of the surgical groups (i.e., with or without omentectomy).

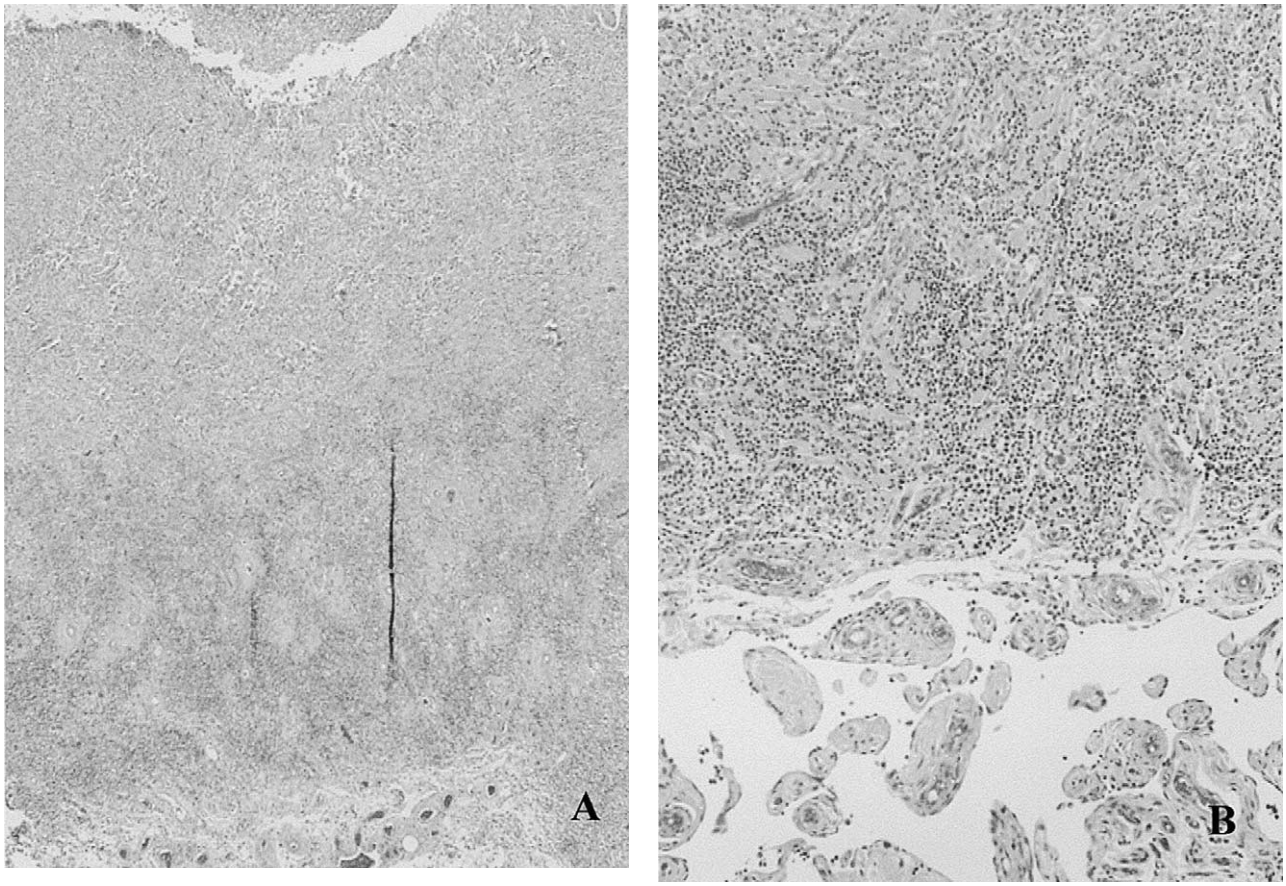

Fig. 2. Histologic examination of grafted area. **A,** Twenty-one days after the surgery, the defect was completely closed by granulation tissue and early fibrosis. Granulation tissue replaced all layers of the gastric wall including the submucosa, muscularis propria, and subserosal fat. (Hematoxylin & eosin stain; ×10.) **B,** Most of the surface of the grafted area was ulcerated. (Hematoxylin & eosin stain; ×10.) Fibrovascular adhesions were seen at the serosal surface.

RESULTS

All of the animals survived and thrived over the 3-week postoperative period. They gained weight (preoperative weight, 320.6 ± 5.5 g; 3 weeks postoperative weight, 371.5 ± 5.9 g) and had no evidence of perforation or clinical signs of leakage. In all instances, animals resumed regular food intake on the day of the surgical manipulation. At autopsy no macroscopic signs of perforation or material disruption were observed. Indeed, the graft area was incorporated into the gastric wall and was distinguished from the normal stomach only by the presence of suture material (Fig. 1). In the first group of animals in which no omentectomy was performed, the omentum was covering the grafted area in five (83.3%) of six rats. However, no adhesions were observed in the abdominal cavity or covering the stomach in four (66.6%) of six animals within the second group of animals in which an omentectomy was performed. The inferior pole of the spleen was covering the stomach in one of the remaining rats, whereas a short segment of small intestine was partially covering the graft in the other animal.

In all 12 animals, histologic examination revealed that the defect was completely closed by granulation tissue and early fibrosis. The granulation tissue replaced all layers of the gastric wall including the submucosa, muscularis propria, and subserosal fat (Fig. 2, *A*). Although most of the luminal surface of the grafted areas remained ulcerated (Fig. 2, *B*), early regeneration of mucosa was seen at the periphery of the former defect (Fig. 3). Foreign body giant cell reaction was present but limited to the areas of suture. Fibrovascular adhesions were present at the serosal surface.

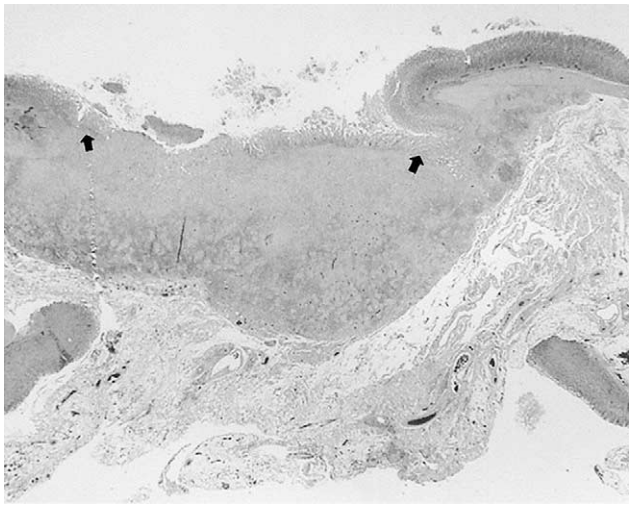

Fig. 3. Low-power overview of grafted area. The defect was completely closed by granulation tissue and early fibrosis. Granulation tissue replaced all layers of the gastric wall including the submucosa, muscularis propria, and subserosal fat. Early regeneration of the mucosa was seen at the periphery of the former defect (*arrows*). Foreign body giant cell reaction was present but limited to the areas of suturing. Fibrovascular adhesions were present at the serosal surface. (Hematoxylin & eosin stain; ×1.)

DISCUSSION

The principal finding reported here is that a double-layer patch of porcine-derived SIS served as a bioscaffold for regeneration of gastric tissue in a rat model of gastric perforation. Twenty-one days after implantation, granulation tissue had replaced the material in all layers of the gastric wall. Moreover, normal gastric mucosa was seen emerging at the periphery of the former defect.

Porcine-derived SIS is an extracellular matrix used in tissue-engineering experiments to create de novo dura mater, urinary bladder, blood vessels, tendons, and so forth.[10–16] Once extracted from the porcine intestines, the matrix is rinsed in water and peracetic acid, and freeze-dried to lyse any cells and eliminate degradation products associated with the matrix.[8] Compositional analysis has shown that the material consists predominantly of collagen types I, III, and IV,[8] with lesser quantities of proteoglycans, glycosaminoglycans,[17] glycoproteins,[18] and growth factors.[19] Interestingly, the bioactive basic fibroblast growth factor-2 (FGF-2) and transforming growth factor-β (TGF-β) are retained in the matrix following sterilization procedures,[19] and this bioactivity remains after prolonged storage at room temperature.[20] Basic FGF is normally present in the gastric mucosa and is a potent stimulator of angiogenesis,[21] accelerating healing of experimental gastric and duodenal ulcers in rats.[22,23] TGF-β is also involved in protecting the mucosa by turning off the proliferation of epithelial cells once they have left the crypts and glands.[21] Although the presence of these growth factors in the matrix might explain the findings observed in our study, information is lacking regarding the existence of other growth factors that are also

Table 1. Experimental applications of SIS matrix in the gastrointestinal tract

Reference	Species	Protocol	Findings
Chen and Badylak[31]	Dogs (n = 23)	Partial small bowel defect (7 × 3 cm)	Three animals died postoperatively due to leakage; autopsy of remaining animals at different time intervals; histologic evaluation showed mucosal layer, smooth muscle, collagen, and serosa; architecturally, layers were not organized
	Dogs (n = 4)	Small intestine interposition of SIS tube	One animal had partial obstruction; three had leakage from anastomotic site within the first week after surgery
Badylak et al.[32]	Dogs (n = 15)	5 × 3 cm esophageal defect or complete circumferential segmental defect (5 cm)	Scaffolds used for repair of defect reabsorbed completely within 60 days; histologic examination showed skeletal muscle, organized collagenous connective tissue, and squamous epithelium; segmental defect repair showed stricture within 45 days
Kini et al.[33]	Humans (n = 14)	Gastrojejunostomy reinforced with 10 × 2.5 cm SIS membrane in patients undergoing gastric bypass	Three patients developed stenosis at the anastomotic site; follow-up 87 days

crucial in maintaining epithelial integrity (e.g., epidermal growth factor, TGF-α, and trefoil factors).

On xenotransplatation of SIS, cellular infiltration and neovascularization are observed, and the matrix is rapidly remodeled into host tissue with site-specific structural properties.[24] The rate of degradation of the scaffold has been reported to range from 4 to 12 weeks[25,26]; as much as 90% of the material may be reabsorbed by as early as 4 weeks after implantation.[25–27]

The relatively fast absorption and healing rate observed in this project must be put into context—that is, all experiments were performed in normal rats with no signs of hypoalbuminemia or nutritional deficits. Animal studies have shown that perioperative malnutrition is associated with compromised anastomosis healing. For example, rats fed a protein-depleted diet have significantly lower colonic anastomosis bursting pressures than animals exposed to a standard diet.[28] Parenteral nutrition and immunosuppressant drugs administered perioperatively to rats also affect intestinal healing.[29,20] Multivariate analysis of patients undergoing bowel resection or bypass anastomosis of the small or large intestine have shown that use of corticoids, bowel obstruction, peritonitis, chronic obstructive pulmonary disease, serum albumin levels, and intraoperative blood transfusion are risk factors associated with anastomosis dehiscence.[2] In our study, animals received standard rat chow throughout the study, and no evidence of weight loss or malnutrition was observed perioperatively. Further studies examining the healing effectiveness of SIS under such conditions are warranted.

Despite increased clinical interest concerning the use of prosthetic materials in gastrointestinal surgery, few studies have assessed the potential uses of SIS in the gut (Table 1). Of the studies that we are aware of, one has used SIS for small bowel restoration[31] and one for esophageal repair.[32] Recently, an SIS membrane was used to reinforce a gastrojejunostomy in patients undergoing gastric bypass surgery.[33] Taken together, these findings show that SIS may act as an effective scaffolding agent for restoration of gastrointestinal tissue and may offer protection in high-risk anastomoses.

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