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ORIGINAL ARTICLE

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Using porcine small intestinal submucosa in intestinal regeneration

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Abstract Small intestinal submucosa (SIS) is an unusual tissue that promotes constructive tissue remodeling when applied as a xenogeneic material. The aim of our experimental study was to assess its effectiveness in intestinal regeneration. Twenty white New Zealand rabbits were anesthetized and underwent celiotomy. A 6-cm antimesenteric incision was created at the jejunal segment. An elliptical SIS graft measuring 6 cm long and 2 cm wide was sutured to the jejunal defect as a patch graft. Thirteen living rabbits were divided into groups of three and the grafts were harvested at postoperative weeks 2, 4, and 6. The obtained specimens were evaluated for gross and histologic appearance. In morphometric examination, in the 2, 4, and 6 weeks groups, the diameters of grafted intestines were larger than preoperatively by 50%, 25%, and 25% respectively; also the grafts had contracted to 0%, 25%, and 50% of their original sizes respectively. At the end of 2 weeks, the grafts were intact without evidence of epithelial regeneration. By 4 weeks, intestinal tissue regeneration was started, and epithelial coverage of the grafts was detected. The grafts were covered with a complete intestinal mucosa at 6 weeks. Remarkable regeneration marked fibroplasia, angiogenesis, and mild mononuclear cell infiltration had also occurred throughout the grafts at 6 weeks. Porcine SIS appeared an effective biodegradable scaffold, facilitating regeneration of intestinal tissue. These results suggest that SIS may be useful to increase the mucosal surface of intestine and may provide a new substance for short gut syndrome in the future.

Keywords Tissue engineering · Small intestinal submucosa · Extracellular matrix · Intestine

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Introduction

Short bowel syndrome is a disease featuring an overall bowel surface deficit that prevents a standard nutritional intake by the patient. To improve bowel absorption or increase small bowel surface, many medical and surgical attempts have been devised [6, 7, 8, 9, 14, 15, 19, 29, 30, 33]. The goals of surgical therapy in short bowel syndrome are to slow transit time and increase the area of absorption. None of the operations for treatment of short bowel syndrome is sufficiently safe and effective to recommend their routine use. Operations should be performed only on selected patients to achieve specific goals. Finally, small bowel transplantation is still a novel therapy and a highly morbid procedure [1, 26].

Some authors have initiated investigation in tissue engineering and in the use of some biomaterials as a way of providing novel small intestinal tissue [2, 12, 16, 23, 24, 28]. This field is still in its infancy, but early investigations appear promising. Small intestinal submucosa (SIS) is a relatively acellular collagen-based matrix from which extensive in situ tissue remodeling has been demonstrated in animal models. It has been used as a scaffold for regeneration of a variety of tissue, including urinary bladder [10, 21, 31], abdominal wall [25], blood vessels [3, 21], tendons [4], durameter [13], bone [27], and esophagus [5]. These findings, ease of obtaining SIS, and the technical simplicity of free grafts, led us to an attempt using SIS free graft as a scaffold for intestinal regeneration. In this study, we

evaluated the effectiveness of SIS in intestinal regeneration in the rabbit model.

Materials and methods

Preparation of SIS

SIS was prepared as previously outlined in detail by Badylak et al. [3]. In brief, sections of porcine jejunum were harvested and immediately placed in normal saline solution. Sections were then cut into 10-to-15-cm lengths and the mesenteric tissues were initially removed from the segment of the small intestine. The segment was then inverted and the superficial portions of the tunica mucosa, including the epithelium and lamina propria, were removed by gentle abrasion using a longitudinal wiping motion with a scalpel handle and saline-moistened gauze. A moderately dense layer of collagen, specifically identified as the stratum compactum of the basilar tunica mucosa, remained as the surface layer of the graft material (Fig. 1). The segment was then reverted to its original orientation and the tunica serosa and tunica muscularis were removed from its outer surface by similar mechanical abrasion. The remaining thin (0.1-to-0.2-mm thick), whitish, translucent tube actually consisted of the tunica submucosa with attached stratum compactum and muscularis mucosa of the tunica mucosa. The stratum compactum that originally contacted the more superficial luminal mucosa was now luminal surface of the graft. The SIS graft was then rinsed with sterile normal saline and stored in refrigerated 0.05% gentamicin used as a graft material. Storage time for the graft materials ranged from 2 to 5 days.

Surgical procedure

White rabbits of the New Zealand strain (1.9–2.3 kg, 15–18 months of age) handled in compliance with the Experimental Research Center of Çukurova University, Turkey were used for all experiments. Each rabbit was anesthetized with 50 mg/kg ketamine and 6 mg/kg xylazine given intramuscularly. Each rabbit was shaved, prepared with povidone iodine, and draped. An upper midline incision was made and a jejunal segment located 40 cm from the Treitz was isolated. A 6-cm antimesenteric incision was created at the isolate jejunal segment. An elliptical SIS graft measuring 6 cm long and 2 cm wide was oriented with the stratum compactum surface facing the intestinal luminal surface. The SIS patch was sutured to the jejunal defect as a patch graft using a continuous running 6/0 PDS suture and reinforced with interrupted 5/0 PDS sutures. After completing the patch, the site was tested for leaks by compressing intraluminal fluids against the suture line.

Each animal received intraoperative antibiotics (ceftriaxone 100 mg/kg bw intramuscularly), and postoperative antibiotics (ceftriaxone, 100 mg/kg bw intramuscularly) once a day for 7 days. The animals were not given food for 48 h after surgery. Hydration was maintained by oral administration of Ringer lactate 5% dextrose solutions. The animals then were allowed to eat soft food, followed by return to normal diet on day 5.

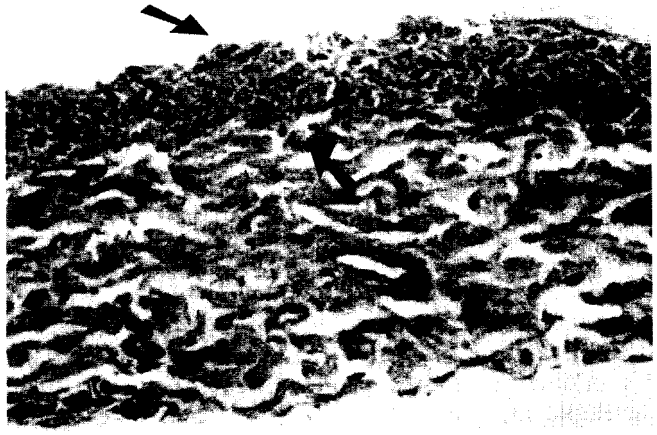

Fig. 1 Histologic examination of the small intestinal submucosa (SIS) graft material at the time of implantation showed the luminal surface to consist of the muscularis mucosa and "stratum compactum" (between two *arrowheads*). (Original magnification ×400, H&E.)

Follow-up and experimental design

Seven animals died from anastomotic leak-related complications and sepsis within postoperative 12 days. At necropsy, some tearings in the suture lines were observed in these cases. Peritonitis secondary to leakage from the anastomotic patch site was found in all rabbits. Thirteen living rabbits were assigned to one of three experimental groups. The experimental period was 2 weeks for group 1 ($n=4$), 4 weeks for group 2 ($n=4$), and 6 weeks for group 3 ($n=5$). All rabbits underwent relaparotomy at the end of their experimental periods. At the laparotomy, graft size and luminal size (diameter of the patched jejunal segment) was measured and then excised for histopathological examination. Intestinal continuity was reestablished with 6/0 PDS running suture. Excised bowel segment was placed in 10% neutral buffered formalin for histologic examination. The formalin-fixed tissues later were embedded in paraffin, sectioned at 6-μm thickness, and stained with H&E and Masson's trichrome.

Results

Macroscopic examination

At the relaparotomy, mild-to-moderate adhesions were noted around this patched area in all animals. These adhesions in the surrounding area made the identification more difficult. The patch graft sites were visible in all rabbits relaparotomized up to 4 weeks after the first surgery. On animals that were evaluated at 6 weeks, the patched area would have been difficult to identify by simple observation. The suture line was used to mark the patch area. At 2 weeks, group I demonstrated no decrease in graft and luminal size. The SIS graft size and patched area contracted to 25% of the initial area at 4 weeks (group 2) and 50% at 6 weeks (Table 1).

Microscopic examination

Histologically, at 2 weeks, the SIS graft was intact without evidence of epithelial regeneration (Fig. 2). Grafts showed a prominent vascular proliferation (neovascularization) (Fig. 2). An infiltration of mononuclear cells and early fibroblastic proliferation were also clearly present in the area of the patch (Fig. 2). By 4 weeks, grafts showed that the remodeled wall contained a mucosal columnar epithelial layer without Goblet cells, sheets of fibroblast, and a serosal covering (Fig. 3). The grafts were also infiltrated completely by well-formed blood vessels and fibroblasts (Fig. 3). Mononuclear cell infiltration was clear, especially around the

Table 1 Morphometric examination results. *SIS* small intestinal submucosa

	SIS size (cm)		Luminal size (cm)
	Length	Width	
Preoperative	6	2	2.1 ± 0.3
Group 1	5.8 ± 0.7	2.1 ± 0.2	3.2 ± 0.3
Group 2	4.6 ± 0.3	1.5 ± 0.16	2.65 ± 0.38
Group 3	3.1 ± 0.4	1.2 ± 0.24	2.45 ± 0.2

Fig. 2 At 2 weeks, small intestinal submucosa (SIS) patch was intact without evidence of epithelial regeneration. Note the dense vascular and fibroblastic proliferation. (Original magnification ×100 H&E.)

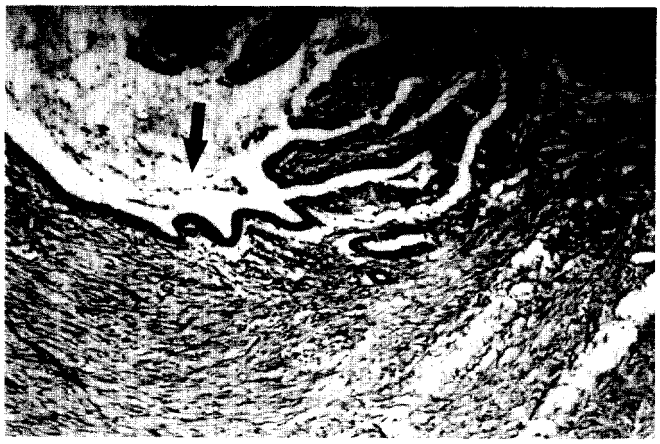

Fig. 3 Small intestinal submucosa (SIS) patch showing a mucosal columnar epithelial layer at postoperative 4 weeks. Vascular and fibroblastic proliferations continuing at the SIS graft at postoperative week 4. (Original magnification ×100 H&E.)

Fig. 4 At 6 weeks, small intestinal submucosa (SIS) graft completely epithelialized with a columnar epithelial cell layer, including goblet cells and villus-like configuration of native tissue. An organized arrangement of collagen fibers formed the entire thickness of the graft. (Original magnification ×100 Masson's trichrome.)

newly formed blood vessels (Fig. 3). By 6 weeks, lumen of SIS completely regenerated to the small intestinal mucosa, including villus like configuration and Goblet cells of native tissue (Fig 4). Well-organized blood vessels persisted and well-organized collagen fibers and scattered fibroblast were interspersed among the individual collagen fibers (Fig 4).

Discussion

Biomaterials play a critical role in the engineering of new functional tissue for the replacement of lost or malfunctioning tissue. They provide a temporary scaffolding to guide new tissue growth and organization. A variety of biomaterials, which can be classified into three types—naturally derived (e.g., collagen, alginate), acellular (e.g., bladder mucosa and small intestinal submucosa), and synthetic polymers (e.g., polyglycolic acid, polylactic acid, and polylactic-coglycolic acid)—have proved to be useful in reconstruction of a number of tissues in animals models [18].

Generally, the ideal biomaterial should be biocompatible, promote cellular interaction and tissue development, and posses proper mechanical and physical properties. Acellular tissue matrices are collagen-rich matrices that are prepared by the removal of cellular components from tissues. SIS, consisting primarily of extracellular matrix material, is prepared by mechanically removing selected portions of the

mucosa and the external muscle layers and then lysing resident cells with hypotonic washes. Numerous studies have shown that this biomaterial is capable of inducing host tissue proliferation, remodeling, and regeneration of tissue structures following implantation in a number of in vivo environments, including lower urinary tract, blood vessels, abdominal wall, tendons, duramater, esophagus, and bone [3, 4, 5, 10, 13, 20, 21, 25, 27, 29]. Angiogenesis, cell migration, differentiation, and the deposition of extensive extracellular matrix have been associated with this regenerative process. The mechanism by which cellular and tissue remodeling develops in response to the SIS consists mainly of extracellular matrix. The SIS materials consist of naturally occurring extracellular matrix that has been shown to be rich in components that support angiogenesis, such as fibronectin; glycosaminoglycans, including heparin; several collagens, including types I, III, IV, and V; and angiogenic factors, such as basic fibroblast growth factor (FGF-2) and vascular endothelial cell growth factor (VEGF) [17, 22, 32]. SIS has also been shown to be nonimmunogenic, as evidenced by the performance of over 1,000 cross-species transplants with no evidence of rejection and by formal direct immunogenic challenge testing that has not elicited a significant response [11].

The model used in this study allowed preliminary evaluation of SIS as a scaffold for the creation of neointestine. SIS grafts were totally replaced by organized collagen tissue by 2 weeks (Fig. 2). Host remodeling occurred primarily from mononuclear and fibroblastic cellular invasion. Histologically, these findings imply that the cellular reaction represents a fibrovascular healing type reaction (Fig. 2). Histologically, columnar epithelium completely covered the SIS patch graft by 4 weeks implantation (Fig. 3). At 6 weeks of regeneration, grafts consisted of well-organized mucosal and submucosal tissues with a complete columnar epithelial cell layer, including goblet cells and villus-like configuration (Fig. 4). These findings are not surprising. It has been shown that SIS is an excellent substrate for epithelial cell growth. This epithelium can serve as a new surface for luminal absorption.

- There was an approximately 25% decrease in the size of grafts at 4 weeks. These grafts contracted to 50% of the initial size at 6 weeks (Table 1). It is logical that the amount of scaffold material at the graft site should affect the time to scaffold degradation. The relationship of scaffold size to rate of degradation and remodeling remained 50% in this study.
- The current therapy for short bowel syndrome is limited. Vacanti and others [2, 12, 16, 23, 24, 28] have initiated investigations in tissue engineering and in the use of other biomaterials as a way of providing novel small intestinal tissue.

The purpose of this study was to evaluate the feasibility of using SIS as a scaffold for small intestinal regeneration. Porcine SIS appeared an effective biodegradable scaffold, facilitating regeneration of intestinal tissue. These results suggest that SIS may be useful to increase the mucosal surface of intestine and may provide a new substance for short gut syndrome in the future. Future studies are needed to focus on the functional aspect of the regenerated small bowel in addition to finding ways to improve to cellular histology and architectural organization that occurs during the remodeling process.

In conclusion, with further studies, SIS may remain a viable option for a scaffold for small intestinal regeneration.

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