Understanding Roles of Porcine Small Intestinal Submucosa in Urinary Bladder Regeneration: Identification of Variable Regenerative Characteristics of Small Intestinal Submucosa

Hsueh-Kung Lin, PhD,¹ Shirley Yezdi Godiwalla, MD,¹ Blake Palmer, MD,¹ Dominic Frimberger, MD,¹ Qing Yang, MS,¹ Sundar V. Madihally, PhD,² Kar-Ming Fung, MD, PhD,³ and Bradley P. Kropp, MD, FAAP, FACS¹

Neuropathic bladders are the result from damages to the central or peripheral nervous system, and ultimately may require surgical reconstruction to increase bladder volumes and to reduce the risk of damages to the kidneys. Surgical reconstruction through bladder augmentation has traditionally been practiced using a segment of the ileum, colon, or stomach from the patient through enterocystoplasty. However, the use of gastrointestinal segments can lead to serious adverse consequences. Porcine small intestinal submucosa (SIS), a xenogeneic, acellular, biocompatible, biodegradable, and collagen-based bioscaffold is best known to encourage bladder regeneration without *ex vivo* cell seeding before implantation in various experimental and preclinical animal models. Although it has been demonstrated that SIS supports bladder cell growth *in vitro*, and SIS-regenerated bladders are histologically and functionally indistinguishable from normal functional tissues, clinical utilization of SIS for bladder augmentation has been hampered by inconsistent preclinical results. Several variables in SIS, such as the age of pigs, the region of the small intestine, and method of sterilization, can have different physical properties, biochemical characteristics, inflammatory cell infiltration, and regenerative capacity due to cellular responses *in vitro* and *in vivo*. These parameters are particularly important for bladder regeneration due to its specific biological function in urine storage. Clinical application of SIS for surgical bladder reconstruction may require graft materials to be prepared from a specific region of the small intestine, or to be further formulated or processed to provide uniform physical and biochemical properties for consistent, complete, and functional bladder regeneration.

Introduction

BLADDER AUGMENTATION, also known as augmentation cystoplasty, is a reconstructive surgery to increase the reservoir capacity and decrease the pressure within the urinary bladder when the bladder experiences various pathological conditions. Conditions such as impairment of bladder outlet afferent and/or efferent signaling can result in pathological development of neuropathic bladders. Neuropathic bladders may result from damages to the central nervous system (stroke, spinal cord injury, meningocele, or amyotrophic lateral sclerosis). Defects in peripheral nerves as the result of diabetes, alcoholism, or vitamin B₁₂ deficiency neuropathies, or a combination of central and peripheral

nervous system disorders (Parkinson's disease, multiple sclerosis, or syphilis), also can result in neuropathic bladders. Furthermore, patients with such conditions as chronic obstructive bladder damage, incontinence, interstitial cystitis, and other chronic inflammatory diseases also may benefit from surgical bladder augmentation. Under these pathological conditions, the bladder progresses to decreased capacity, decreased compliance, increased pressure, and sometimes incomplete emptying.

Main purposes of bladder augmentation are to increase the bladder capacity and to protect the kidneys from damage due to high bladder pressures. For example, surgical bladder reconstruction has been shown to provide long-term satisfaction and success in upper-tract safety and continence in

¹Department of Urology, The Children's Hospital of Oklahoma, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma.

²Department of Chemical Engineering, Oklahoma State University, Stillwater, Oklahoma.

³Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma.

patients with spinal cord injury.¹ Enterocystoplasty using a segment from the ileum, colon, or stomach of the patient is a common practice for bladder augmentation.^{2,3} However, the use of gastrointestinal segments may lead to adverse consequences, including nutritional and electrolyte alterations, peritoneal adhesions, abscesses, enteric fistulae, excessive mucus production, bladder rupture, bacterial colonization, stone formation, or cancer.^{4–7} To minimize these consequences, the field of tissue engineering attempts to develop alternative biological scaffolds to replace the gastrointestinal segment. These scaffolds range from natural collagen-based matrices^{8,9} to biodegradable synthetic polymers, such as polyglycolic acid, polylactic acid, or poly D,L-lactic-co-glycolic acid (PLGA).^{10–12} Clinical outcomes from using these natural and synthetic biomaterials remain unknown or inconclusive.

Small intestinal submucosa (SIS) is a native collagen-based extracellular matrix (ECM) of submucosal layer of intestinal wall after the tunica mucosa and the serosa muscularis are mechanically removed from the porcine small intestine.¹³ SIS has been demonstrated to be biocompatible and has no adverse response in cross-species transplantations.^{14,15} This biomaterial has been used as a supporting scaffold to repair a variety of tissues, including arterial and venous tissues,^{13,16,17} tendon,^{13,17} and wound-closure¹⁸ in various animal models.^{19,20} SIS has been approved by the Food and Drug Administration (FDA) for a variety of clinical applications, including urethral sling for stress urinary incontinence, fistula plugs for rectal, urethral, and vaginal fistulae, inguinal and ventral hernias, vaginal defect repairs for cystocele, rectoceles, or enteroceles, and Peyronie's disease repairs. For urinary tract reconstruction, SIS is one of the most thoroughly studied scaffolds in both experimental and preclinical models.^{21–25} In bladder reconstruction, SIS acts as a scaffold, and relies on the ECM to orchestrate the regenerative capacity of host bladder cells without prior *ex vivo* cell seeding.^{21–23,26} The elimination of the *ex vivo* cell seeding step using cells derived from the patients reduces the risk of introducing artificially manipulated cells into the hosts. Long-term follow-up studies in a dog bladder augmentation model show that SIS grafts can be remodeled and replaced by the host tissue. SIS-regenerated bladders are histologically indistinguishable from native bladder tissues with urothelium, smooth muscle, vascular structure, and peripheral nerves, and are functionally normal.^{8,21,24} However, bladder augmentation using porcine SIS has demonstrated inconsistent results in the same preclinical model, and SIS cannot be applied in human clinical cases for surgical bladder augmentation until these variations have been determined and corrected.

In this review, we describe our experiences using SIS as a bladder-wall substitute in rodent and canine bladder augmentation models, and propose possible sources of inconsistent preclinical results. We demonstrate that physical and biochemical characteristics as well as regenerative properties are different along the entire small intestine for bladder regeneration. Reliable bladder regeneration will be achieved once appropriate physical characteristics and biological responses are identified and optimized. Consistent and successful bladder regeneration will then be obtainable using SIS for surgical bladder augmentation in human patients.

SIS Supports Three-Dimensional Bladder Cell Growth in Cultures

ECM-based porcine SIS is a naturally derived three-dimensional bioscaffold. SIS supports cell proliferation, differentiation, migration, and spatial organization. A variety of cell types, including NIH Swiss mouse 3T3 fibroblasts, NIH 3T3/J2 fibroblasts, primary human fibroblasts, primary human keratinocytes, and bone marrow-derived mesenchymal stem cells (MSCs), have been studied for their growth behaviors and patterns on SIS.^{27–29} All studies on the variety of cell types from different species have shown that porcine SIS supports cell attachment, growth, and differentiation *in vitro*. In addition, in contrast to two-dimensional tissue culture systems, cells can use the three-dimensional bioscaffold and migrate into the interconnective porous structure of SIS.

It also has been demonstrated that SIS is compatible with urinary bladder urothelial cells and smooth muscle cells (SMCs), as well as endothelial cells and nerve cells *in vitro*. These studies have provided important information regarding interactions between SIS and cells.^{30–33} Human urothelial cells and bladder SMCs attached to SIS and proliferated into uninterrupted multiple layers, as shown in Figure 1A and B, respectively. Similarly, SIS supports the growth of primary cultures of urothelial cells and SMCs from multiple species, including rabbits, pigs, dogs, and humans.^{30,34–36} In addition, SIS supported the growth of bone marrow-derived MSCs and their differentiation into SMC-like phenotype in cultures,^{28,29} as well as growth and migration of human embryonic stem cells cocultured with bladder urothelial cells and SMCs.³⁷ In contrast, Feil *et al.* reported that commercial SIS induces cytotoxicity in primary cultures of human urothelial cells; the authors concluded that SIS may not be suitable for the construction of urothelial cell-SIS grafts *in vitro*.³⁸ The dramatic differences of urothelial cell responses to SIS may result from several different parameters of porcine SIS, as discussed below.

SIS also provides a tool to study cell-cell interactions on the three-dimensional bioscaffold. Using a urothelial and bladder SMC coculture system, close spatial relationships and interactions between these two cell types enhanced cell proliferation and migration on SIS (Fig. 1C), while cells maintained the urothelial cell-specific phenotype with AE1/AE3 expression and the SMC-specific phenotype with α -smooth muscle actin (α -SMA) expression (Fig. 1D). Consistently, in cocultured primary cultures of human epidermal cells and NIH 3T3/J2 fibroblasts on an SIS bioscaffold, Lindberg *et al.* demonstrated a distinctive spatial orientation of the two cell types, and enhanced migration and invasiveness of fibroblasts in the presence of epidermal cells within the matrix.²⁷

To determine whether SIS would support angiogenesis and nerve regeneration during the process of tissue regeneration, endothelial cells—including human microvascular endothelial cells (HMECs) and human umbilical vein endothelial cells^{27,39,40}—as well as rat peripheral nerve Schwann cells³³ have been studied for their attachment, migration, and growth on SIS. These endothelial and peripheral nerve cells were able to grow to confluence on the surface of SIS, to penetrate the SIS, and to express cell type-specific growth factors. For example, HMECs attached (Fig. 1E) and

FIG. 1. Culture of human bladder cells on small intestinal submucosa (SIS). Human bladder cells were seeded on commercial SIS and cultured for a period of 7 days. **(A)** Masson's trichrome stained section of primary cultures of urothelial cells (brown) grown on the mucosal surface of SIS (blue) *in vitro*. **(B)** Masson's trichrome staining of primary cultures of human bladder smooth muscle cells (SMCs) (brown) grown on the mucosal side of SIS (blue). **(C)** Masson's trichrome staining of the sandwich technique: urothelial cells were grown on the mucosal side of SIS with bladder SMCs seeded on the opposite side (serosal side) of SIS. **(D)** Double immunohistochemical staining of cells grown in layered coculture on SIS: urothelial cells stain positive for cytokeratin marker AE1/AE3 (red) and grow in multiple layers with some early polarity on top of several layers of α -actin-positive SMCs (brown), which are beginning to penetrate the SIS. **(E)** Masson's trichrome staining of human microvascular endothelial cell (HMEC) (red) grown on the mucosal surface of SIS (blue) in cultures.

FIG. 2. Histological evaluation of bladder regeneration in a rat bladder augmentation model. Immunohistochemical and Masson's trichrome staining of regenerating bladders was performed at day 56 postaugmentation **(A)**. Detection of uroplakin-positive immunoreactivity specifically in superficial and deeper layers of urothelial cells. **(B)** Diffused and homogeneous ZO-1-positive immunoreactivity throughout the urothelium. **(C)** Development of smooth muscle bundles demonstrated by α -SMA-positive staining. **(D)** Capillary blood vessel formation shown by CD31-positive immunohistochemistry. **(A)** and **(B)** were adapted from Roth *et al.*⁴³

proliferated on SIS (Fig. 1F). More importantly, SIS supports blood vessel ingrowth using the *in ovo* chicken embryo chorioallantoic membrane vascular assay.⁴¹ The ability of SIS to support endothelial and nerve cell attachment and proliferation may implicate the significance of the biomaterial in rapid remodeling responses *in vivo* after SIS graft implantation for wound repair, angiogenesis, and axonal regeneration.

In summary, SIS is biocompatible with a variety of cell types, including urothelial cells, bladder SMCs, endothelial cells, and peripheral nerve cells. All of these cell types are actively involved in functional bladder regeneration. These *in vitro* studies also suggest that SIS may contain a mixture of bioactive molecules, including cell adhesion factors, chemotactic factors, mitogenic factors, and angiogenic factors. These factors are important to support bladder regeneration and angiogenesis *in vivo*. As a three-dimensional bioscaffold, SIS is suitable for *in vitro* studies of cellular responses to the ECM and cell–cell interactions between and among urothelial, smooth muscle, endothelial, and peripheral nerve cells. These studies will be valuable for evaluating the presence and availability of bioactive molecules in porcine SIS for urinary bladder regeneration before it is used for clinical applications.

Functional Regeneration of Urinary Bladder Using SIS

SIS has been extensively studied as a bioscaffold in bladder regeneration in a variety of preclinical models, including rodent,^{42,43} rabbit,⁴⁴ canine,⁴⁵ and porcine.⁴⁶ Generally, animals were subjected to partial cystectomy (40–60 percent of bladder wall) followed by augmentation with single layer SIS grafts. In a rat bladder augmentation model, complete urothelization was observed within 14 days after augmentation.⁴³ Uroplakin expressions in the form of cytoplasmic immunoreactivity was noted in the single layer of the regenerating urothelium on day 2; on day 56 postoperation, uroplakin was homogeneous in the superficial and deeper layers of urothelial cells (Fig. 2A). ZO-1-positive immunoreactivity in the form of cytoplasmic staining also was demonstrated in the single layer of regenerating urothelium by postoperative day 2, and the expression of ZO-1 in the regenerating urothelium was homogeneous throughout the urothelial regeneration (Fig. 2B). In addition, histological analysis demonstrated the development of smooth muscle bundles (Fig. 2C) and capillary formation by CD31-positive immunohistochemistry (Fig. 2D). Long-term histological evaluation shows that SIS-regenerated urinary bladders contain all of the normal bladder wall architecture and no evidence of inflammation.

In an SIS-augmented dog bladder regeneration model, completed urothelium and smooth muscle regeneration was observed at a 15-month follow-up.²² Nerve regeneration was

evidenced by immunohistochemistry of afferent nerves and by functional characterization of contractile activity for efferent nerves. Urodynamic studies demonstrated normal compliance and contractile activity in SIS-augmented dog bladders.²³ No diverticular formation, bladder calculi, mucus, or any urinary infection were observed. In addition, the SIS grafts did not result in significant contraction in the large animal model. Similar regeneration processes and results also were demonstrated in other experimental models, such as rabbits and pigs, with inner transitional epithelium, outer collagen with fibroblasts and muscular bundles, along with new vessel formation and nerve regeneration.^{44,46} Results from these experimental and preclinical models suggest that porcine SIS is a viable biomaterial for bladder augmentation, and can be an alternative to the use of intestine for autologous bladder wall reconstruction.

Results from preclinical studies demonstrated that SIS grafts become indistinguishable from native bladder tissues after regeneration. To confirm that SIS is disintegrated during the process of tissue regeneration, radiolabeled SIS was first established by administering 10 μ Ci of ¹⁴C-proline intravenously to piglets for a period of 23 weeks. Radiolabeled SIS was harvested and used for bladder augmentation in a canine model, and the ¹⁴C-labeled SIS was used to quantify the rate of porcine SIS degradation *in vivo*.⁴⁷ At 3 months postaugmentation, the reconstructed bladders contained less than 10 percent of the original ¹⁴C radioactivity at the time of implantation. These results suggest that the SIS biomaterial provides a temporary scaffold with a rapid host tissue remodeling, and that degraded SIS was eliminated via urine when the biomaterial was used for urinary bladder wall repair and reconstruction.

Differential Regenerative Properties of SIS in Preclinical Bladder Augmentation Models

After the initial success in SIS-mediated bladder regeneration observed in rodent and canine models, inconsistent regeneration was persistent and reported in these models using commercial SIS obtained from COOK Biotech (Lafayette, IN). Many possibilities may contribute to inconsistent bladder regeneration using COOK SIS, including sterilization methods, the age of pigs, and the region of the small intestine.

Sterilization methods have been studied to determine their effects on biocompatibility and biodegradation of SIS. Grimes *et al.* compared ethylene oxide, gamma irradiation, and e-beam irradiation, and concluded that these processes increase the rate of hydrolytic degradation of SIS.⁴⁸ When SIS extracts prepared after these sterilization methods were applied to L929 mouse fibroblasts to assess the biocompatibility of degraded products, all methods caused increases in both cell proliferation and cellular protein production. SIS sterilized by ethylene oxide had the greatest effect at initial

FIG. 4. Differential regenerative properties of SIS prepared from different segments of the small intestine. Bladder regeneration was followed in the dog bladder augmentation model following partial cystectomy. Bladders were harvested at 10 weeks following augmentation. Masson's trichrome staining was performed for histological evaluation. **(A)** Completed bladder regeneration was observed after augmentation distal SIS. **(B)** Histological presentations of urothelial and smooth muscle layers in distal SIS-augmented dog bladder. **(C)** Graft shrinkage and stone formation in bladder augmented with proximal SIS. **(D)** Histological features of proximal SIS-augmented bladder showing incomplete regeneration with lack of urothelium and smooth muscle development.

FIG. 3. Dog bladder regeneration using e-beam sterilized and lyophilized SIS. Using the dog bladder augmentation model, regenerative bladders were harvested at 10 weeks following SIS augmentation. **(A)** Graft shrinkage and deep calcification (arrow) deposit on the surface using lyophilized SIS. **(B)** Graft shrinkage and long bone-like structure (arrow) using e-beam sterilized SIS. **(C)** Scar development and central calcification (arrow) augmented with lyophilized SIS. **(D)** Lack of muscle regeneration and transmural bone formation in the dog regenerative bladder augmented with lyophilized SIS.

phases, whereas unsterilized SIS had a slower, but more prolonged influence on cell growth and cellular protein expression.⁴⁸

The composition and three-dimensional matrix structure of SIS were compared after peracetic acid and ethylene oxide sterilization.⁴⁹ Microarchitectures were significantly different between these two sterilization methods. Using peracetic acid sterilization, the complex yet open structure of native SIS and the perpendicular fiber orientation of the original tissue are preserved; and processed samples show denser mucosal surfaces, open and porous serosal, and uniform population of fibers. In addition, fibronectin and hyaluronic acid contents are retained in SIS following peracetic acid and ethylene oxide sterilization, whereas levels of transforming growth factor β 1 (TGF β 1) and vascular endothelial growth factor (VEGF) are significantly reduced in disinfected versus unsterilized SIS. Importantly, sulfated glycosaminoglycans (GAGs) and fibroblast growth factor (FGF-2) contents are significantly enriched in peracetic acid-treated SIS.⁵⁰ Sterilized SIS is capable of supporting fibroblast attachment and release of VEGF, and promoting PC12 cell differentiation with increased neurite outgrowth in culture conditions.⁵⁰

The sterilization method has significant impacts on the outcomes of bladder regeneration. Using the dog bladder augmentation model, peracetic acid and ethylene oxide sterilization consistently show better regeneration compared to e-beam sterilization or lyophilized SIS. Graft shrinkage calcification (Fig. 3A), bone formation (Fig. 3B), scar development (Fig. 3C), little muscle regeneration, (Fig. 3D), and elevated inflammation are commonly observed in augmented dog bladders using e-beam sterilized and lyophilized SIS.

The age of pigs from which SIS is made also can have a significant impact on the biocompatibility and regenerative capacity of SIS. Tottey *et al.* demonstrated that SIS harvested from differently aged pigs has distinct differences in the structural, mechanical, and compositional properties.⁵¹ Using peracetic acid-treated SIS,⁴² mechanical strength, elasticity, and growth factor contents are lower in SIS from young (3-week-old) pigs than those prepared from older animals. In addition, degradation products of SIS from all ages of pigs are chemotactic for perivascular stem cells, whereas SIS-degraded products obtained from older animals are most potent in inducing cell proliferation.

To investigate regenerative properties of SIS derived from different regions of the small intestine, small intestines were harvested from 3-year-old sows and divided into proximal and distal regions. Distal SIS is defined as segments of ileum isolated from 300 to 400 cm proximal to the ileocecal valve; proximal SIS is defined as the first 100 cm of proximal jejunum taken 50 cm distal to the duodenum. After mechanically removing cellular structures from mucosal and serosal sides of the small intestine, acellular SIS was sterilized with 0.1% peracetic acid in absolute ethanol and stored in double-distilled water.⁴³ Using the canine bladder augmentation model, bladder regeneration was studied using distal and proximal SIS as scaffolds (graft size 7 $\times$ 10 cm) following partial cystectomy. Completed bladder regeneration was observed at 10 weeks; after augmentation, distal SIS was used (Fig. 4A), and the regenerated bladder demonstrated the presence of urothelial and smooth muscle layers (Fig. 4B). In contrast, proximal SIS-augmented dog bladder showed shrinkage and stone formation in graft areas (Fig. 4C) and histological features demonstrated

incomplete regeneration with lack of urothelium and/or smooth muscles (Fig. 4D). Superior regenerative properties of distal SIS were similarly observed in the rat bladder augmentation model compared to proximal SIS.⁵²

Inconsistent regenerative characteristics of SIS also have been observed in tissues other than bladder. In a primate abdominal wall-repair model, commercial SIS-based implants demonstrated scar tissue formation and contraction, coincident with mesh pleating, and were characterized by immediate and significant cellular and humoral inflammatory responses.⁵³ In contrast, in dog abdominal wall-defect repair, SIS grafts were incorporated into adjacent tissue structures and developed well-organized smooth muscle and collagenous connective tissue based on immunohistochemical staining and clinical observations.^{18,20} The loss of structural and biological integrity from one or a combination of the above variables may hinder normal wound-healing and tissue-regeneration processes.

Physical Characteristics of SIS

Structural features of biomaterials are also important parameters underlying cellular attachment, infiltration, proliferation, differentiation, and eventual incorporation of the biomaterials into healing host tissues.^{54–57} Results from bladder regeneration studies in animal models suggest that variations in physical characteristics throughout the porcine small intestine may contribute differential regenerative properties. For example, biomaterials' permeability to urine may have detrimental effects on urothelial cell growth, interfering with re-epithelization of regenerative bladders,⁵⁸ altering inflammatory responses, and resulting in incomplete bladder regeneration.

Several structural features of SIS have been analyzed and reported. Using small-angle light scattering to quantify the gross fiber structure, Sacks and Gloeckner demonstrated that the structure of SIS is uniform throughout the length of the small intestine and always longitudinally aligned, or oriented parallel to the long axis of the intestine.⁵⁹ An asymmetric mechanical longitudinal and circumferential strain level coupling test suggests strong mechanical interactions on a fiber level.

To better understand the possible relationship between the heterogeneous nature and differential regenerative property of SIS, physical characteristics (including compliance, elastic modulus, and burst pressure) were quantitatively measured and compared between distal and proximal SIS prepared from 3-year-old sows. Surface architecture was first examined by scanning electron microscopy (SEM).⁶⁰ No significant differences were observed in the serosal sides of proximal and distal SIS, except in the distribution of fibers on the surface (Fig. 5A). In addition, there was no significant difference in the mucosal side of the proximal and distal SIS. However, the mucosal side of the SIS membranes showed less porous architecture relative to the corresponding serosal side. Tensile properties were quantified by the stress-strain behavior at a monotonic loading of 10 mm/min until failure in phosphate-buffered saline rehydrated SIS. The tensile strain at break was higher in the distal than the proximal SIS. Young's modulus values were used to measure the stiffness of SIS, and results showed that distal SIS was more elastic (elastic modulus was 3 MPa) than proximal SIS (elastic modulus was 8 MPa).

The bladder undergoes cyclical loading conditions due to filling and voiding, behaving like viscoelastic materials that store and dissipate energy, producing hysteresis. A full description of the mechanical response of SIS requires viscoelastic behavior to know the amount of stress that would accumulate due to each expansion and relaxation. In this regard, commercially available SIS was subjected to a constant step tensile strain and allowed to relax for certain time.⁶¹ The load limits were predetermined by the stress-strain curves to ensure that the samples were within the break stress and break strain region. SIS showed an increase in peak stress in successive stages, behaving like a strain hardening material similar to many biological tissues (Fig. 5B). On the contrary, synthetic 50:50 PLGA membranes that have been explored in bladder regeneration showed a decrease in peak stresses in successive stages, behaving as strain-softening material (Fig. 5C). These distinctions help in understanding why SIS is superior to synthetic structures. In addition to the load capacity of SIS biomaterial, minimum urinary diversion with appropriate bladder filling (or mechanical signaling) during the early phases of tissue remodeling process is important for complete bladder regeneration.⁶²

SIS permeability to urea, a small, uncharged polar nitrogen-containing molecule in the urine, was assessed using a custom-made chamber.⁶⁰ Results demonstrated that distal SIS prepared from 3-year-old sows had the least permeability to urea as compared to proximal SIS (Fig. 5E, F). In addition, consistent to SEM micrographs of surface architecture, permeability in each sample was direction-dependent, that is, the mucosal-to-serosal direction was less permeable compared to the serosal-to-mucosal direction in all SIS analyzed.⁶⁰ Analyses of physical characteristics support the hypothesis that distal samples are more elastic and less permeable to urea relative to other samples, and provide more consistent and reliable bladder regeneration.

Biochemical Properties of SIS

In addition to physical characteristics, biochemical compositions of naturally derived biomaterials can determine outcomes of tissue regeneration. Growth promoting ECM constituents have been identified and studied in SIS. Polyanoglycans (GAGs) are important components of the ECM, consist of multiple species (including hyaluronic acid, heparin, heparan sulfate, chondroitin sulfate A, and dermatan sulfate), and have been found in SIS. In contrast, two GAG species that are common to other tissues with large basement membrane ECM components, keratan sulfate and chondroitin sulfate C, were not detected in SIS extracts.⁶³ The importance of the presence or absence of these GAGs at this time is unknown and informational only.

Contents of growth factors in SIS also have been qualitatively and quantitatively determined by biochemical, immunohistochemical, and biological techniques. Enzyme-linked immunosorbent assay and Western blot analysis showed that SIS contains FGF-2 and VEGF, but not a closely related endothelial cell mitogen, platelet-derived growth factor.^{64,65} When immunohistochemistry was applied in frozen SIS sections to detect the presence of various growth factors, Hurst *et al.* reported the presence of immunolabeled heparan sulfate proteoglycans, FGF-2, and VEGF in SIS, whereas TGF- β was barely detectable above the background.⁶⁶ It should be noted that although the presence of

growth factor proteins have been identified in the SIS material, it is unknown whether these proteins contribute in any way to the regenerative process.

To determine whether biological molecules present in SIS are functionally active, SIS was acid hydrolyzed and different sizes of proteins were fractionated based on molecular weights. A fraction containing 5- to 16-kDa proteins possesses a potent chemoattractant activity for primary murine adult liver, heart, and kidney endothelial cells *in vitro*, and promotes vascularization when the fraction was included in Matrigel plugs, and subsequently implanted subcutaneously in mice.⁶⁷ Biological activities of FGF-2 and VEGF isolated from SIS also were assessed by bioassays. The inclusion of the anti-FGF-2 or anti-VEGF neutralizing antibody blocked fibroblast proliferation or endothelial cell (HMEC) tube formation in a three-dimensional fibrin-based angiogenesis assay, respectively.^{64,65} Similar to results obtained from immunohistochemistry, identification and biological activity of TGF- β and its related proteins in SIS remain inconclusive.⁶⁴

Inflammatory Responses in SIS-Augmented Bladders

It is well understood that the normal process of wound healing involves infiltration of inflammatory cells. What is unknown at this junction is that what type of an inflammatory response occurs when an organ or tissue is regenerating. Therefore, although inflammation can be perceived to have a negative impact on tissue regeneration, we present the following information on inflammation to try to better understand the mechanisms of the inflammatory response as it relates to tissue regeneration.

Porcine SIS has been used in many experimental models and human subjects as a xenogeneic scaffolding material without clinical rejection. In animals and human subjects, recipients produce antibodies specific for SIS after implantation without developing clinical signs of rejection, wound infection, hernia recurrence, or interfering SIS uptake or remodeling.^{68,69} To study SIS-elicited inflammatory responses in chemokines and cytokines expression, SIS was used as an immune adjuvant and injected intraperitoneally and subcutaneously into mouse models. Aachoui and Ghosh demonstrated that SIS affects cytokine and chemokine microenvironments by upregulating interleukin-4 (IL-4) and CD30 ligand as well as activating chemotactic factors LIX and KC (neutrophil chemotactic factors), MCP-1 (monocyte chemotactic factors), MIP 1- α (macrophage chemotactic factor), lymphotactin, and growth factors like M-CSF.⁷⁰

To classify SIS-activated immune responses *in vivo*, SIS was subcutaneously implanted into mice and the SIS grafts were analyzed histologically. SIS implants activate an acute inflammatory response, but gradually diminish, and the SIS grafts ultimately become indistinguishable from native tissue.⁷¹ Both Th1 and Th2 immune responses have been reported in animal models receiving SIS grafts. Allman *et al.* showed that SIS grafts activate Th2-restricted immune responses with an increase in IL-4, but absence of interferon- γ (INF- γ) without signs of rejection.^{71,72} In contrast, when Palmer *et al.* studied subsets of human helper T cell activation and differentiation *in vitro* models, SIS partially suppresses Th1 cell expansion and secretion of IL-12 and INF- γ in a TGF- β -dependent manner; however, Th1 cell expansion and INF- γ secretion were restored by addition of

FIG. 5. Structural and physical characteristics of SIS. **(A)** Under scanning electron microscopy, no significant differences were observed in the serosal sides of proximal and distal SIS, except in the distribution of fibers on the surface. The mucosal side of the SIS membranes showed less porous architecture relative to the corresponding serosal side. Relaxation behavior under different stages of ramp and hold tests for **(B)** SIS and **(C)** synthetic 50:50 poly D,L-lactic-co-glycolic acid membranes. Each stage was normalized to the origin by subtracting the values at the end of the previous stage. **(B)** and **(C)** were adapted from Mirani *et al.*⁶¹

recombinant IL-12.⁷³ In contrast to results reported by Allan *et al.*, SIS completely abolishes the generation of Th2 cells *in vitro*; and the suppression of Th cell activation by SIS involved the induction of Th cell apoptosis through a caspase-dependent pathway(s), but independent of TGF- β , Fas ligand (FasL), or galectin family members.⁷⁴ Other

studies showed that SIS appears to have no effects on proinflammatory cytokines (IL-6, IL-1 β , TNF- α) or NLRP3 expression, but promotes both Th1 and Th2 responses under different conditions.⁷⁰ Sterilization methods such as freeze-drying and gas sterilization may have significant impacts by activating or inactivating unidentified bioactive

FIG. 6. Differential inflammatory responses elicited by distal and proximal SIS. Using the rat bladder regeneration model, infiltrated inflammatory cells were distinguished by single-cell-type specific histological staining. **(A–C)** Proximal SIS recruits significantly higher numbers of eosinophils at early and late phases of bladder augmentation as compared to distal SIS. **(D–F)** Sustained recruitment of macrophages is observed in late phases of distal SIS-augmented bladder versus proximal SIS-augmented bladder (Fig. 6B). Arrows denote location where high-power inset images ($\times 60$) were taken. **(A–F)** were adapted from Ashley *et al.*⁵²

molecules in SIS and eliciting differential biological responses.

We further demonstrated that SIS-activated immune responses are dependent upon different regions of the small intestine in the rat bladder augmentation model.⁵² Using cell type-specific histological staining, proximal SIS recruited significantly higher numbers of eosinophils and mast cells at early and late phases of bladder augmentation, respectively, as compared to distal SIS (Fig. 6A–C). In addition, sustained recruitment of polymorphonuclear cells and macrophages were observed in early and late phases of distal SIS-augmented bladder, respectively, versus proximal SIS-augmented bladder (Fig. 6D–F). Expression and secretion of immune cytokines by these cells need to be further studied in combination with results obtained from microarray and bioinformatics to understand inflammatory and other molecular events that lead to differential regeneration results when the bladder is augmented with distal and proximal SIS.⁵²

Conclusions

Patients with neuropathic bladders resulted from different etiologies may require surgical reconstruction to improve bladder compliance and reduce the risk of kidney damage. Enterocystoplasty has successfully been used to treat a variety of bladder dysfunctions in human patients who require surgical bladder reconstruction. Due to possible long-term adverse effects using gastrointestinal segments for bladder augmentation, other natural and synthetic biomaterials have been intensively studied as alternative replacements.

SIS prepared from the pig small intestine is a biocompatible, xenogeneic, native collagen-based tissue matrix bioscaffold. SIS has shown to be a promoter for tissue regeneration without prior *ex vivo* seeding of *in vitro* expanded cells. As a biologic scaffold, SIS stimulates repairs of damaged or diseased tissues, and regenerates tissues with a structure and function similar to original ones. Functional evaluations of SIS in experimental and preclinical bladder augmentation models have been very useful for assessing scaffold properties and establishing *in vivo* feasibility before clinical applications.

To minimize variables potentially introduced by *ex vivo* cell seeding or biochemically preconditioned bioscaffolds, and to maximize regenerative properties of SIS, we propose that SIS can be biochemically and physically modified to provide a uniform structure. This modification might be especially important in urinary bladder regeneration to reduce urine permeability, enhance urothelial cell growth and differentiation, and improve complete bladder regeneration in patients who require surgical bladder reconstruction.

Acknowledgment

The authors wish to thank Cook Biotech, Inc. for providing the porcine SIS for the experiments.

Disclosure Statement

No competing financial interests exist.

References

- Gurung, P.M., Attar, K.H., Abdul-Rahman, A., Morris, T., Hamid, R., and Shah, P.J. Long-term outcomes of augmentation ileocystoplasty in patients with spinal cord injury: a minimum of 10 years of follow-up. *BJU Int* **109**, 1236, 2011.
- Scales, C.D., Jr., and Wiener, J.S. Evaluating outcomes of enterocystoplasty in patients with spina bifida: a review of the literature. *J Urol* **180**, 2323, 2008.
- Mitsui, T., Tanaka, H., Moriya, K., Matsuda, M., and Nonomura, K. Outcomes of lower urinary and bowel function in meningo-myelocele patients with augmentation enterocystoplasty. *Spinal Cord* **46**, 432, 2008.
- Kurzrock, E.A. Pediatric enterocystoplasty: long-term complications and controversies. *World J Urol* **27**, 69, 2009.
- Hensle, T.W., and Gilbert, S.M. A review of metabolic consequences and long-term complications of enterocystoplasty in children. *Curr Urol Rep* **8**, 157, 2007.
- Robertson, W.G., and Woodhouse, C.R. Metabolic factors in the causation of urinary tract stones in patients with enterocystoplasties. *Urol Res* **34**, 231, 2006.
- Qiu, H., Kordunskaya, S., and Yantiss, R.K. Transitional cell carcinoma arising in the gastric remnant following gastrocystoplasty: a case report and review of the literature. *Int J Surg Pathol* **11**, 143, 2003.
- Kropp, B.P. Small-intestinal submucosa for bladder augmentation: a review of preclinical studies. *World J Urol* **16**, 262, 1998.
- Kropp, B.P., and Cheng, E.Y. Bioengineering organs using small intestinal submucosa scaffolds: *in vivo* tissue-engineering technology. *J Endourol* **14**, 59, 2000.
- Vance, R.J., Miller, D.C., Thapa, A., Haberstroh, K.M., and Webster, T.J. Decreased fibroblast cell density on chemically degraded poly-lactic-co-glycolic acid, polyurethane, and polycaprolactone. *Biomaterials* **25**, 2095, 2004.
- Nakanishi, Y., Chen, G., Komuro, H., Ushida, T., Kaneko, S., Tateishi, T., *et al.* Tissue-engineered urinary bladder wall using PLGA mesh-collagen hybrid scaffolds: a comparison study of collagen sponge and gel as a scaffold. *J Pediatr Surg* **38**, 1781, 2003.
- Atala, A. Tissue engineering in urology. *Curr Urol Rep* **2**, 83, 2001.
- Badylak, S.F., Lantz, G.C., Coffey, A., and Geddes, L.A. Small intestinal submucosa as a large diameter vascular graft in the dog. *J Surg Res* **47**, 74, 1989.
- Paterson, R.F., Lifshitz, D.A., Beck, S.D., Siqueira, T.M., Jr., Cheng, L., Lingeman, J.E., *et al.* Multilayered small intestinal submucosa is inferior to autologous bowel for laparoscopic bladder augmentation. *J Urol* **168**, 2253, 2002.
- Murphy, F., and Corbally, M.T. The novel use of small intestinal submucosal matrix for chest wall reconstruction following Ewing's tumour resection. *Pediatr Surg Int* **23**, 353, 2007.
- Lantz, G.C., Badylak, S.F., Coffey, A.C., Geddes, L.A., and Sandusky, G.E. Small intestinal submucosa as a superior vena cava graft in the dog. *J Surg Res* **53**, 175, 1992.
- Lantz, G.C., Badylak, S.F., Coffey, A.C., Geddes, L.A., and Blevins, W.E. Small intestinal submucosa as a small-diameter arterial graft in the dog. *J Invest Surg* **3**, 217, 1990.
- Clarke, K.M., Lantz, G.C., Salisbury, S.K., Badylak, S.F., Hiles, M.C., and Voytik, S.L. Intestine submucosa and polypropylene mesh for abdominal wall repair in dogs. *J Surg Res* **60**, 107, 1996.
- Prevel, C.D., Eppley, B.L., Summerlin, D.J., Sidner, R., McCarty, M., and Badylak, S.F. Small intestinal submucosa: utilization as a wound dressing in full-thickness rodent wounds. *Ann Plast Surg* **35**, 381, 1995.

20. de Castro Marques, A.I., Hipwell, F., and Yool, D.A. The use of porcine small-intestinal submucosa for abdominal wall reconstruction—a clinical case. *J Small Anim Pract* **50**, 619, 2009.
21. Vaught, J.D., Kropp, B.P., Sawyer, B.D., Rippy, M.K., Badylak, S.F., Shannon, H.E., *et al.* Detrusor regeneration in the rat using porcine small intestinal submucosal grafts: functional innervation and receptor expression. *J Urol* **155**, 374, 1996.
22. Kropp, B.P., Rippy, M.K., Badylak, S.F., Adams, M.C., Keating, M.A., Rink, R.C., *et al.* Regenerative urinary bladder augmentation using small intestinal submucosa: urodynamic and histopathologic assessment in long-term canine bladder augmentations. *J Urol* **155**, 2098, 1996.
23. Kropp, B.P., Sawyer, B.D., Shannon, H.E., Rippy, M.K., Badylak, S.F., Adams, M.C., *et al.* Characterization of small intestinal submucosa regenerated canine detrusor: assessment of reinnervation, *in vitro* compliance and contractility. *J Urol* **156**, 599, 1996.
24. Pope, J.C.I., Davis, M.M., Smith, E.R., Jr., Walsh, M.J., Ellison, P.K., Rink, R.C., *et al.* The ontogeny of canine small intestinal submucosa regenerated bladder. *J Urol* **158**, 1105, 1997.
25. Shalhav, A.L., Elbahnasy, A.M., Bercowsky, E., Kovacs, G., Brewer, A., Maxwell, K.L., *et al.* Laparoscopic replacement of urinary tract segments using biodegradable materials in a large-animal model. *J Endourol* **13**, 241, 1999.
26. Sutherland, R.S., Baskin, L.S., Hayward, S.W., and Cunha, G.R. Regeneration of bladder urothelium, smooth muscle, blood vessels and nerves into an acellular tissue matrix. *J Urol* **156**, 571, 1996.
27. Lindberg, K., and Badylak, S.F. Porcine small intestinal submucosa (SIS): a bioscaffold supporting *in vitro* primary human epidermal cell differentiation and synthesis of basement membrane proteins. *Burns* **27**, 254, 2001.
28. Shukla, D., Box, G.N., Edwards, R.A., and Tyson, D.R. Bone marrow stem cells for urologic tissue engineering. *World J Urol* **26**, 341, 2008.
29. Zhang, Y., Lin, H.K., Frimberger, D., Epstein, R.B., and Kropp, B.P. Growth of bone marrow stromal cells on small intestinal submucosa: an alternative cell source for tissue engineered bladder. *BJU Int* **96**, 1120, 2005.
30. Campodonico, F., Benelli, R., Michelazzi, A., Ognio, E., Toncini, C., and Maffezzini, M. Bladder cell culture on small intestinal submucosa as bioscaffold: experimental study on engineered urothelial grafts. *Eur Urol* **46**, 531, 2004.
31. Zhang, Y., Kropp, B.P., Lin, H.K., Cowan, R., and Cheng, E.Y. Bladder regeneration with cell-seeded small intestinal submucosa. *Tissue Eng* **10**, 181, 2004.
32. Mondalek, F.G., Lawrence, B.J., Kropp, B.P., Grady, B.P., Fung, K.M., Madhally, S.V., *et al.* The incorporation of poly(lactic-co-glycolic) acid nanoparticles into porcine small intestinal submucosa biomaterials. *Biomaterials* **29**, 1159, 2008.
33. Su, Y., Zeng, B.F., Zhang, C.Q., Zhang, K.G., and Xie, X.T. Study of biocompatibility of small intestinal submucosa (SIS) with Schwann cells *in vitro*. *Brain Res* **1145**, 41, 2007.
34. Baumert, H., Mansouri, D., Fromont, G., Hekmati, M., Simon, P., Massoud, W., *et al.* Terminal urothelium differentiation of engineered neoureter after *in vivo* maturation in the "omental bioreactor". *Eur Urol* **52**, 1492, 2007.
35. Zhang, Y., Frimberger, D., Cheng, E.Y., Lin, H.K., and Kropp, B.P. Challenges and a larger bladder replacement with cell-seeded and unseeded small intestinal submucosa (SIS) grafts in a subtaotal cystectomy model. *BJU Int* **98**, 1100, 2006.
36. Pariente, J.L., Kim, B.S., and Atala, A. *In vitro* biocompatibility assessment of naturally derived and synthetic biomaterials using normal human urothelial cells. *J Biomed Mater Res* **55**, 33, 2001.
37. Lakshmanan, Y., Frimberger, D., Gearhart, J.D., and Gearhart, J.P. Human embryoid body-derived stem cells in co-culture with bladder smooth muscle and urothelium. *Urology* **65**, 821, 2005.
38. Feil, G., Christ-Adler, M., Maurer, S., Corvin, S., Rennekampff, H.O., Krug, J., *et al.* Investigations of urothelial cells seeded on commercially available small intestine submucosa. *Eur Urol* **50**, 1330, 2006.
39. Hodde, J.P., Record, R.D., Tullius, R.S., and Badylak, S.F. Retention of endothelial cell adherence to porcine-derived extracellular matrix after disinfection and sterilization. *Tissue Eng* **8**, 225, 2002.
40. Yang, B., Zhou, L., Sun, Z., Yang, R., Chen, Y., and Dai, Y. *In vitro* evaluation of the bioactive factors preserved in porcine small intestinal submucosa through cellular biological approaches. *J Biomed Mater Res A* **93**, 1100, 2010.
41. Mondalek, F.G., Ashley, R.A., Roth, C.C., Kibar, Y., Shakir, N., Ihnat, M.A., *et al.* Enhanced angiogenesis of modified porcine small intestinal submucosa with hyaluronic acid-poly(lactide-co-glycolide) nanoparticles: From fabrication to preclinical validation. *J Biomed Mater Res A* **94**, 712, 2010.
42. Ashley, R.A., Palmer, B.W., Schultz, A.D., Woodson, B.W., Roth, C.C., Routh, J.C., *et al.* Leukocyte inflammatory response in a rat urinary bladder regeneration model using porcine small intestinal submucosa scaffold. *Tissue Eng Part A* **15**, 3241, 2009.
43. Roth, C.C., Bell, C.H., Woodson, B., Schultz, A.D., Palmer, B.W., Frimberger, D., *et al.* Temporal differentiation and maturation of regenerated rat urothelium. *BJU Int* **103**, 836, 2009.
44. Ayyildiz, A., Akgul, K.T., Huri, E., Nuhoglu, B., Kilicoglu, B., Ustun, H., *et al.* Use of porcine small intestinal submucosa in bladder augmentation in rabbit: long-term histological outcome. *ANZ J Surg* **78**, 82, 2008.
45. Kropp, B.P., Cheng, E.Y., Lin, H.K., and Zhang, Y. Reliable and reproducible bladder regeneration using unseeded distal small intestinal submucosa. *J Urol* **172**, 1710, 2004.
46. Caione, P., Capozza, N., Zavaglia, D., Palombaro, G., and Boldrini, R. *In vivo* bladder regeneration using small intestinal submucosa: experimental study. *Pediatr Surg Int* **22**, 593, 2006.
47. Record, R.D., Hillegonds, D., Simmons, C., Tullius, R., Rickey, F.A., Elmore, D., *et al.* *In vivo* degradation of ¹⁴C-labeled small intestinal submucosa (SIS) when used for urinary bladder repair. *Biomaterials* **22**, 2653, 2001.
48. Grimes, M., Pembroke, J.T., and McGloughlin, T. The effect of choice of sterilisation method on the biocompatibility and biodegradability of SIS (small intestinal submucosa). *Biomed Mater Eng* **15**, 65, 2005.
49. Hodde, J., Janis, A., Ernst, D., Zopf, D., Sherman, D., and Johnson, C. Effects of sterilization on an extracellular matrix scaffold: part I. Composition and matrix architecture. *J Mater Sci Mater Med* **18**, 537, 2007.
50. Hodde, J., Janis, A., and Hiles, M. Effects of sterilization on an extracellular matrix scaffold: part II. Bioactivity and matrix interaction. *J Mater Sci Mater Med* **18**, 545, 2007.

51. Tottey, S., Johnson, S.A., Crapo, P.M., Reing, J.E., Zhang, L., Jiang, H., *et al.* The effect of source animal age upon extracellular matrix scaffold properties. *Biomaterials* **32**, 128, 2011.
52. Ashley, R.A., Roth, C.C., Palmer, B.W., Kibar, Y., Routh, J.C., Fung, K.M., *et al.* Regional variations in small intestinal submucosa evoke differences in inflammation with subsequent impact on tissue regeneration in the rat bladder augmentation model. *BJU Int* **105**, 1462, 2010.
53. Sandor, M., Xu, H., Connor, J., Lombardi, J., Harper, J.R., Silverman, R.P., *et al.* Host response to implanted porcine-derived biologic materials in a primate model of abdominal wall repair. *Tissue Eng Part A* **14**, 2021, 2008.
54. An, B., Desrochers, T.M., Qin, G., Xia, X., Thiagarajan, G., Brodsky, B., *et al.* The influence of specific binding of collagen-silk chimeras to silk biomaterials on hMSC behavior. *Biomaterials* **34**, 402, 2013.
55. Grover, C.N., Cameron, R.E., and Best, S.M. Investigating the morphological, mechanical and degradation properties of scaffolds comprising collagen, gelatin and elastin for use in soft tissue engineering. *J Mech Behav Biomed Mater* **10**, 62, 2012.
56. Masaeli, E., Morshed, M., Rasekhian, P., Karbasi, S., Karambalaie, K., Karamali, F., *et al.* Does the tissue engineering architecture of poly(3-hydroxybutyrate) scaffold affects cell-material interactions? *J Biomed Mater Res A* **100**, 1907, 2012.
57. Stabenfeldt, S.E., and LaPlaca, M.C. Variations in rigidity and ligand density influence neuronal response in methylcellulose-laminin hydrogels. *Acta Biomater* **7**, 4102, 2011.
58. Davis, N.F., Callanan, A., McGuire, B.B., Flood, H.D., and McGloughlin, T.M. Evaluation of viability and proliferative activity of human urothelial cells cultured onto xenogenic tissue-engineered extracellular matrices. *Urology* **77**, 1007 e1, 2011.
59. Sacks, M.S., and Gloeckner, D.C. Quantification of the fiber architecture and biaxial mechanical behavior of porcine intestinal submucosa. *J Biomed Mater Res* **46**, 1, 1999.
60. Raghavan, D., Kropp, B.P., Lin, H.K., Zhang, Y., Cowan, R., and Madhally, S.V. Physical characteristics of small intestinal submucosa scaffolds are location-dependent. *J Biomed Mater Res A* **73A**, 90, 2005.
61. Mirani, R.D., Pratt, J., Iyer, P., and Madhally, S.V. The stress relaxation characteristics of composite matrices etched to produce nanoscale surface features. *Biomaterials* **30**, 703, 2009.
62. Boruch, A.V., Nieponice, A., Qureshi, I.R., Gilbert, T.W., and Badylak, S.F. Constructive remodeling of biologic scaffolds is dependent on early exposure to physiologic bladder filling in a canine partial cystectomy model. *J Surg Res* **161**, 217, 2010.
63. Hodde, J.P., Badylak, S.F., Brightman, A.O., and Voytik-Harbin, S.L. Glycosaminoglycan content of small intestinal submucosa: a bioscaffold for tissue replacement. *Tissue Eng* **2**, 209, 1996.
64. Voytik-Harbin, S.L., Brightman, A.O., Kraine, M.R., Waisner, B., and Badylak, S.F. Identification of extractable growth factors from small intestinal submucosa. *J Cell Biochem* **67**, 478, 1997.
65. Hodde, J.P., Record, R.D., Liang, H.A., and Badylak, S.F. Vascular endothelial growth factor in porcine-derived extracellular matrix. *Endothelium* **8**, 11, 2001.
66. Hurst, R.E., and Bonner, R.B. Mapping of the distribution of significant proteins and proteoglycans in small intestinal submucosa by fluorescence microscopy. *J Biomater Sci Polym Ed* **12**, 1267, 2001.
67. Li, F., Li, W., Johnson, S., Ingram, D., Yoder, M., and Badylak, S. Low-molecular-weight peptides derived from extracellular matrix as chemoattractants for primary endothelial cells. *Endothelium* **11**, 199, 2004.
68. Raeder, R.H., Badylak, S.F., Sheehan, C., Kallakury, B., and Metzger, D.W. Natural anti-galactose alpha1,3 galactose antibodies delay, but do not prevent the acceptance of extracellular matrix xenografts. *Transpl Immunol* **10**, 15, 2002.
69. Ansaloni, L., Cambrini, P., Catena, F., Di Saverio, S., Gagliardi, S., Gazzotti, F., *et al.* Immune response to small intestinal submucosa (surgisis) implant in humans: preliminary observations. *J Invest Surg* **20**, 237, 2007.
70. Achoui, Y., and Ghosh, S.K. Extracellular matrix from porcine small intestinal submucosa (SIS) as immune adjuvants. *PLoS One* **6**, e27083, 2011.
71. Allman, A.J., McPherson, T.B., Badylak, S.F., Merrill, L.C., Kallakury, B., Sheehan, C., *et al.* Xenogeneic extracellular matrix grafts elicit a Th2-restricted immune response. *Transplantation* **7**, 1631, 2001.
72. Allman, A.J., McPherson, T.B., Merrill, L.C., Badylak, S.F., and Metzger, D.W. The Th2-restricted immune response to xenogeneic small intestinal submucosa does not influence systemic protective immunity to viral and bacterial pathogens. *Tissue Eng* **8**, 53, 2002.
73. Palmer, E.M., Beilfuss, B.A., Nagai, T., Semnani, R.T., Badylak, S.F., and van Seventer, G.A. Human helper T cell activation and differentiation is suppressed by porcine small intestinal submucosa. *Tissue Eng* **8**, 893, 2002.
74. Palmer, E.M., Baum, L.G., and van Seventer, G.A. Small intestinal submucosa induces loss of mitochondrial integrity and caspase-dependent apoptosis in human T cells. *Tissue Eng* **9**, 307, 2003.

Address correspondence to:
 Bradley P. Kropp, MD, FAAP, FACS
 Department of Urology
 The Children's Hospital of Oklahoma
 University of Oklahoma Health Sciences Center
 920 Stanton L. Young Blvd. WP3150
 Oklahoma City, OK 73104
 E-mail: brad-kropp@ouhsc.edu

Received: February 19, 2013

Accepted: June 6, 2013

Online Publication Date: July 25, 2013