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Urologic tissue engineering with small-intestinal submucosa: potential clinical applications

Abstract Small-intestinal submucosa (SIS) is a unique biomaterial that has been shown to induce tissue-specific regeneration in numerous organ systems. In the urinary tract, animal studies have demonstrated that SIS promotes functional bladder regeneration. Other preliminary studies have suggested that SIS may also be extremely useful for several other types of urologic surgery application where new tissue is needed or reinforcement of native structures is desired. This article reviews past and current work with SIS in the urinary tract and focuses on applications that will likely have future clinical utility.

Key words Tissue engineering · Bladder · Regeneration

Over the last decade there has been an explosion of tissue-engineering research in an effort to provide replacement tissue for the patient with a diseased organ. At present, two different types of tissue-engineering technology are being investigated for the creation of regenerated tissue grafts: in vitro and in vivo technologies. In vitro technology uses biodegradable membranes that are seeded in vitro with primary cultured cells that have been established from a biopsy specimen of the host's native tissue [3, 4, 26]. This composite graft is then placed back in the host for the continuation of the regenerative process.

In vivo tissue-engineering technology involves the placement of a biodegradable material (without cells) in the host that then functions as a scaffold to allow the natural process of regeneration to occur. This process aims at recapitulating the normal embryologic development of the organ of interest. Thus far, investigators have focused their efforts on in vivo technology using a unique biomaterial known as small-intestinal submucosa (SIS). SIS is a xenogenic membrane that is harvested from porcine small intestine, after which the tunica mucosa, serosa, and tunica muscularis are mechanically removed from the inner and outer surfaces of the graft. This results in a collagen-rich membrane that is approximately 0.1 mm thick and is composed mainly of the submucosal layer of the intestinal wall. SIS is unique from other previously used graft materials in that it contains functional growth factors that are likely vital to the regenerative process [33]. SIS has been shown to induce tissue-specific regeneration in numerous tissues, including the aorta, vena cava, heart, ligaments, and skin [5, 6, 22–24, 28]. SIS has also been shown to be nonimmunogenic as evidenced by the performance of over 1000 cross-species transplants with no evidence of rejection and by formal direct immunogenic challenge testing that has not elicited a significant response (Badylak, personal communication) [25].

Initial work with SIS in the urinary tract has focused on augmenting the size of the bladder with regenerated bladder tissue. SIS has been extensively investigated in animal models as a bladder augmentation graft using in vivo tissue-engineering technology. As work has progressed and it has become readily apparent that the induction of functional regenerated bladder tissue can be achieved with SIS, additional urologic applications have begun to be investigated. SIS has been preliminarily studied for use as a corporal body graft, in urethral reconstruction, and as an injectable form for the correction of vesicoureteral reflux and incontinence. These and other potential clinical applications for SIS in the urinary tract are discussed below.

Bladder augmentation

Currently the most common form of bladder augmentation is intestinocystoplasty. Unfortunately,

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several unwanted side effects are associated with ileal intestinocystoplasty, including infections, stones, electrolyte abnormalities, mucus production, spontaneous perforation, and tumor development. For these reasons, alternative methods have been sought, including the use of synthetic and nonsynthetic grafts. The major obstacle to in vivo tissue-engineering technology in the setting of bladder augmentation has been the lack of an appropriate graft material that would act as a suitable scaffold to allow the native bladder to regenerate itself. Synthetic nonbiodegradable biomaterials such as silicone, rubber, polytetrafluoroethylene, and polypropylene have been used in the past but have proved to be unsuccessful because of host foreign-body reactions [2, 7, 8, 21, 31]. As a consequence of the failure of these synthetic nonbiodegradable materials, different types of biodegradable material have since been investigated. In theory, biodegradable grafts would be advantageous over nonbiodegradable materials in that they would allow the host bladder time for regeneration but would then dissolve prior to the onset of any deleterious foreign-body reaction. These materials have been applied experimentally and have shown a marked improvement over nonbiodegradable materials [1]. Biomaterials that are rich in collagen, such as placenta, amnion, and pericardium, have achieved the greatest success [9, 11, 12, 30]. However, although initial results have been encouraging, none of these materials has been found to be suitable for clinical use. The reasons for this are not entirely clear. It can only be speculated from the literature that the long-term results obtained with these materials did not recapitulate the initial results.

In vivo tissue-engineering technology for urinary reconstruction made little progress, if any, over the past 20 years until research with SIS began. The initial research using SIS for urinary bladder augmentation was performed in a rat model [16]. A total of 22 rats underwent a partial cystectomy followed by immediate augmentation with a 1-cm^2 patch of SIS. Rats were then euthanized at various time points ranging from 2 weeks to 11 months. Histology studies revealed that the SIS-regenerated rat bladders contained all three layers of the bladder (urothelium, smooth muscle, and serosa) and was nearly indistinguishable from normal rat bladder at 11 months postaugmentation. This study demonstrated that SIS functioned as an adequate scaffold to allow the native rat bladder to remodel and regenerate itself. To determine whether the SIS-regenerated bladder was functional, subsequent in vitro contractility studies were also performed. These studies confirmed that SIS-regenerated bladder in the rat displayed contractile properties and nerve regeneration similar to that exhibited by the normal rat bladder [32]. These studies provided the first line of evidence that a functional bladder could be achieved with in vivo tissue-engineering technology. They also demonstrated that SIS was uniquely different and more successful than other previously studied biodegradable materials.

Encouraged by these initial results in the rat, investigators conducted additional studies investigating the regenerative potential of SIS in the bladder using in vivo tissue-engineering technology in a long-term, large-animal model. SIS bladder augmentations were performed in 19 dogs after 40% of the bladder had been removed via partial cystectomy [17]. Animals were euthanized at 1–15 months postsurgery. The results of this study demonstrated that at 15 months the SIS-regenerated bladders were urodynamically compliant, with capacities being similar to those exhibited by control dogs. No deleterious side effect or upper-tract change was observed in any of the animals. Histologically, all three layers of the bladder had regenerated. However, the quantity and organization of smooth-muscle fibers differed slightly from that seen in the normal bladder. In vitro bladder-strip contractility studies on the SIS-regenerated portions of the bladder demonstrated that the contractile activity and the expression of muscarinic, adrenergic, and purinergic receptors were similar to those displayed by normal bladder. In addition, SIS-regenerated bladder demonstrated functional nerve regeneration and innervation that was similar to that of normal bladder tissue. Furthermore, in vitro stress/strain compliance studies demonstrated no significant difference between SIS-regenerated bladder and control bladder, both of which were 30-fold more compliant that the original SIS graft material [19].

The above-mentioned work with SIS regenerative bladder augmentations in the dog supports the hypothesis that the native normal bladder can regenerate itself without the complications of graft shrinkage, graft incrustation, or infection. With the addition of current and ongoing work, SIS has become the most thoroughly studied collagen-based biomaterial for bladder augmentation and urinary reconstruction [15–19, 27, 32]. This work has obvious and significant clinical ramifications. Nonetheless, additional work is needed. The cellular mechanisms involved in the bladder-regenerative process promoted by SIS are currently unknown. Further knowledge of the biologic importance of various components of SIS, the growth factors that are involved in the regenerative process, and the cell-to-cell communication that occurs during this process is vital to the full realization of the clinical potential of SIS in the setting of bladder augmentation and replacement.

Urethral reconstruction

In the absence of a sufficient amount of penile skin, reconstruction of the male urethra in patients with complex hypospadias and stricture disease can be a formidable task. The use of free skin grafts and of buccal and bladder mucosa has proved to be successful in providing adequate tissue for urethral replacement but requires harvesting of tissue from a secondary site.

Given SIS's ability to promote bladder regeneration, preliminary investigations have been carried out to determine whether this type of in vivo tissue-engineering technology could also be applied to the urethra.

In 1998, Kropp et al. [20] conducted a study in which SIS was used as an onlay patch graft ($n = 8$) for urethroplasty in rabbits. SIS was compared with full-thickness preputial skin grafts ($n = 8$) and shams (simple urethrotomy and closure, $n = 4$). Animals were euthanized at between 8 and 12 weeks after the procedures. Technically, SIS demonstrated excellent workability. Histologic evaluation demonstrated that SIS promoted urethral regeneration. Regenerated urethra contained three to four layers of stratified columnar urothelium that was indistinguishable from the normal rabbit urothelium. There was also evidence of regeneration of circular smooth muscle underneath the urothelium. This regenerated muscle was contained within an abundant amount of collagen and fibrous connective tissue. Grossly there was no evidence of diverticular formation. In contrast, all grafts in the preputial skin group showed evidence of diverticulum formation.

The results of this pilot study suggest that SIS grafts are feasible for onlay urethroplasty in a short-term animal model. It also appears that SIS-regenerated urethra has clear structural advantages over preputial skin in this model. Further long-term studies certainly need to be conducted to determine the clinical applicability of SIS for urethral reconstructive surgery. However, if future animal and clinical studies demonstrate that SIS is useful in promoting the formation of a permanent neourethra, then SIS could prove to be very valuable in cases in which penile skin is not available. It would represent an "off-the-shelf" graft that would negate the necessity for harvesting of a free graft from another part of the body.

Corporal body

Replacement grafts for defects in the tunica albuginea of the corporal body are necessary in several clinical conditions. In patients with severe penile chordee a grafting procedure on the ventral side of the corporal bodies is preferable to dorsal tuck procedures due to the significant shortening of penile length that results from the latter. Also, in patients with Peyronie's disease, excision of the plaque creates a defect in the tunica albuginea that requires a grafting procedure. Several autologous materials have been used for tunica albuginea grafts, including dermis, vein, tunica vaginalis, and fascia. As is the case in free grafts for urethral reconstruction, these grafts require secondary harvesting of the graft.

Weigel et al. [34] have preliminarily investigated the utility of SIS as a replacement graft in the tunica albuginea. In all, 20 rats underwent implantation of a 7×3-mm SIS graft following elliptical excision of an equal size of tunica from the corporal body. Animals were euthanized at various time points ranging between 1 and 24 weeks postprocedure. There was no evidence of SIS graft shrinkage or contraction at any time point. Histology revealed the occurrence of an initial inflammatory response followed by neovascularization of the graft and incorporation into the native corporal tissue.

These encouraging results have resulted in a clinical pilot study using SIS as a tunica albuginea graft in children with severe penile chordee with or without hypospadias. Thus far, two children have undergone grafting procedures and the results of short-term follow-up have been excellent. SIS was found to be technically easy to work with, provided a watertight graft, and resulted in excellent correction of the chordee. Short-term clinical follow-up has shown no clinical evidence of fibrosis, contraction, or recurrent curvature. There has been no intraoperative or postoperative complication. Further experience and long-term follow-up are required to corroborate these early encouraging results before SIS can be recommended for universal use for tunica albuginea grafting.

Injectable SIS

Endoscopic treatment of vesicoureteral reflux and urinary incontinence has been shown over the last two decades to be technically feasible and effective. Current problems are related not to the procedure itself but rather to the appropriateness of the material injected. Two injectable materials, polytetrafluoroethylene (Teflon) and bovine collagen, have been studied extensively in the clinical setting and have been shown to be efficacious in correcting reflux and incontinence in select patients. Unfortunately, Teflon has been found to migrate from the site of injection, resulting in granuloma formation, and collagen is resorbed and degraded with time. A universally accepted injectable material for urologic use has not yet been identified. Ideally, the perfect substance would be readily obtained, easily injected, nonimmunogenic, nonmigratory, and efficacious over the long term. SIS may represent such a substance.

The first investigation of an injectable form of SIS was performed by Knapp et al. [13] with canine SIS in a porcine bladder model. They documented that a submucosal injection of SIS produced neoconnective tissue composed of spindle-shaped cells at the site of injection. In a canine reflux model, Safir et al. [29] investigated the use of an injectable porcine form of SIS. Reflux was corrected postinjection in five of six dogs. However, reflux recurred to some degree in all animals at the time of euthanasia. Important endoscopic observations made in this pilot study included the following: (1) the SIS formulation used in this pilot study did not form a well-visualized bleb at the site of injection; (2) SIS was noted to leak out of the needle tract after injection; and (3) the surgically created reflux in the dog model is unlike that occurring naturally in the human, which renders difficult the endoscopic correction of reflux in this animal model with any

substance. The first two observations likely relate to the viscosity of the injectable SIS. Despite the relatively poor viscosity and leakage that was observed with this formulation of SIS, histologic examination demonstrated that SIS induced collagen deposition and the ingrowth of de novo spindle-shaped cells that stained positively for smooth-muscle α-actin.

The above-mentioned initial studies using injectable SIS prompted a more recent study in which four different formulations of SIS with superior paste-like characteristics were investigated [10]. The specific processes used to make these formulations are proprietary to the sponsor (Cook Biotech, Inc.), but all paste formulations were mechanically processed in a similar manner. Formulations differed with respect to the age of the SIS source (sow versus slaughtering age) and the sterilization methods used (aseptic processing with or without E-beam irradiation). For elimination of the possible experimental variables of an animal reflux model, injectable SIS was placed submucosally in a normal canine bladder. A total of 12 dogs underwent direct-vision submucosal injection of all 4 SIS formulations and were euthanized at 2 weeks, 6 weeks, 3 months, and 6 months after surgery. Histologic evaluation revealed that de novo smooth-muscle cells appeared as early as at 6 weeks, and by 6 months, early muscle-bundle formation was seen. Grossly, all the formulations showed significant submucosal volume loss ($>50\%$) relative to the amount of SIS originally injected.

Overall, two of the four formulations produced superior results with regard to gross retention of submucosal volume, collagen deposition, and induction of smooth-muscle-cell regeneration. Analysis of the formulations revealed that these two formulations were E-beam-irradiated. This suggests that E-beam irradiation may prolong the smooth-muscle induction in and resorptive process of this matrix. Radiation at a molecular level causes protein denaturing and nucleic acid fragmentation at sterilization doses. Why E-beam irradiation allows better tissue regeneration and volume preservation can only be speculated at this time. It may be secondary to partial collagen cross-linking, thus allowing the matrix to resist absorption. This and other possibilities require further investigation.

The mechanism by which injectable substances correct reflux and/or incontinence has been theorized to rely on the physical material itself as a bulking agent that changes the anatomy of the ureterovesical junction or bladder neck. The results of these preliminary studies suggest that SIS may not only act as a bulking agent but may also be beneficial in its ability to induce new smooth-muscle formation in an area of presumed deficiency. This new muscle formation may be functionally more beneficial than SIS's bulking properties. It is tempting to speculate that the development of this autologous smooth muscle at the injection site is permanent. The exact functional role of this new muscle has not yet been defined and is currently being evaluated.

Other potential uses for SIS in urologic surgery

In general, SIS can be considered for use in any condition in which new tissue or reinforcement of existing native tissue is desired. Several other areas in which SIS may prove to be valuable in urologic surgery are under preliminary study or are being considered for future investigation. These include bladder neck suspension, vaginal wall replacement, renal coverage following partial nephrectomy or renal reconstruction, and use as an interpositional graft following vesicovaginal fistula repair and other types of fistula repair in the urinary tract.

Finally, all of the work carried out to date using SIS in the urinary tract has involved the application of in vivo tissue-engineering technology. The utility of SIS with in vitro technology has yet to be explored. This is an area of active interest in our laboratory, where we have demonstrated that SIS can successfully be seeded in vitro with cultured bladder smooth-muscle and epithelial cells. Subsequent multilayered and differentiated growth of both cell types has been observed [14, 35]. It is unclear whether the use of SIS with in vitro tissue-engineering technology (in which SIS is seeded with cultured cells prior to graft placement in the host) will enhance results previously obtained using SIS with in vivo technology or whether SIS will prove to be better than other synthetic biomaterials that are currently being investigated for use with in vitro technology. Future work will be aimed at answering these important questions.

Conclusions

The future of tissue-engineering technology in the urinary tract is bright. It is clear that regeneration of bladder and other urologic tissues can be achieved using currently available in vivo and in vitro tissue-engineering technologies. The utility of SIS with in vivo technology is encouraging, and studies are under way to evaluate its utility and efficacy with in vitro technology. The clinical application of these technologies is on the horizon; however, caution should be exercised, since several questions remain to be answered prior to their widespread clinical use. One of the most important issues that needs to be addressed is whether regeneration of normal tissue can be accomplished from a pathologic organ. Thus far, all of the animal studies investigating tissue-specific regeneration with both in vivo and in vitro technologies have used animals with a normal organ (bladder). This is clearly not the case in humans, where one needs to harvest cells from a diseased organ or use pathologic tissue as a template for regeneration of new tissue. Further understanding of the regenerative process and the factors that influence the normal growth and function of cells will help us to address this clinically relevant issue. Nevertheless, it is the opinion of the authors that the endless potential of current tissue-engi-

neering techniques in the urinary tract is just now being realized. Future reconstructive urologic surgery will surely involve novel techniques based on the research currently being performed in numerous laboratories.

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