

Xenogenic Extracellular Matrices as Potential Biomaterials for Interposition Grafting in Urological Surgery

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Abbreviations and Acronyms

BSM = bladder submucosal matrix

ECM = extracellular matrix

GAG = glycosaminoglycan

IL = interleukin

NSS = nephron sparing surgery

SIS = small intestinal submucosa

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Purpose: The field of tissue engineering focuses on developing strategies for reconstructing injured, diseased, and congenitally absent tissues and organs. During the last decade urologists have benefited from remodeling and regenerative properties of bioscaffolds derived from xenogenic extracellular matrices. We comprehensively reviewed the current literature on structural and functional characteristics of xenogenic extracellular matrix grafting since it was first described in urological surgery. We also reviewed the clinical limitations, and assessed the potential for safe and effective urological application of extracellular matrix grafting in place of autogenous tissue.

Materials and Methods: We performed literature searches for English language publications using the PubMed® and MEDLINE® databases. Keywords included “xenogenic,” “extracellular matrix” and “genitourinary tract applications.” A total of 112 articles were scrutinized, of which 50 were suitable for review based on clinical relevance and importance of content.

Results: Since the mid 1990s xenogenic extracellular matrices have been used to successfully treat a number of pathological conditions that affect the upper and lower genitourinary tract. They are typically prepared from porcine organs such as small intestine and bladder. These organs are harvested and subjected to decellularization and sterilization techniques before surgical implantation. Bioinductive growth factors that are retained during the preparation process induce constructive tissue remodeling as the extracellular matrix is simultaneously degraded and excreted. However, recent documented concerns over durability, decreased mechanical strength and residual porcine DNA after preparation techniques have temporarily hampered the potential of extracellular matrices as a reliable replacement for genitourinary tract structures.

Conclusions: Extracellular matrices are a useful alternative for successfully treating a number of urological conditions that affect the genitourinary tract. However, clinical concerns regarding mechanical limitations and biosafety need to be addressed before their long-term role in reconstructive urological surgery can be clearly established.

Key Words: tissue engineering; extracellular matrix; reconstructive surgical procedures; intestine, small; urinary bladder

A broad range of benign and malignant medical conditions can lead to destruction or obliteration of the genitourinary tract in a focal or multi-segmental manner. The field of tissue engineering fo-

cuses on developing strategies for reconstructing injured, diseased, and congenitally absent tissues and organs. This exciting development has progressed considerably during the last 2

decades, as exemplified by the different xenogenic biomaterials that urologists are using on a day-to-day basis ([Appendix 1](#)). Hitherto, reconfigured autogenous bowel segments have been the mainstay of functional reconstruction for the lower urinary tract since the early 1900s.¹ Although bowel is used commonly, it is not without its own specific complications such as recurrent calculus formation, recurrent resistant urinary tract infections and metabolic abnormalities.² A safe, strong xenogenic material is a potential alternative option.

We review the evolution and clinical limitations of xenogenic ECM grafting since it was first described in urological surgery during the mid 1990s.³ We examined the structural and functional characteristics of different xenogenic ECMs and assessed their potential to be safely applied to the genitourinary tract in everyday urological practice in place of autogenous tissue.

CONSTITUENTS AND PREPARATION OF XENOGENIC ECMS

The major constituents found in mammalian ECMs include collagen, glycoproteins (such as fibronectin and laminin), GAGs and growth factors. These constituents provide structural, functional, adhesive and stimulatory functions to the surrounding cells, enabling them to survive and proliferate ([Appendixes 2 and 3](#)).⁴ Naturally the transition phase of an ECM scaffold from intact mammalian tissue to viable donor xenograft material requires several processing steps. Initially native tissue is manually separated from unwanted tissue structures and tissue decellularization is achieved through various combinations of physical, chemical and enzymatic treatments.⁵ These methods disrupt the cell membrane and facilitate extraction of intracellular remnants.

During the decellularization process it is of paramount importance to preserve as many mechanical and biological properties of the donor ECM as possible. Disruption of collagen architecture can decrease the mechanical strength of the prepared scaffold, removal of GAGs adversely affect its viscoelastic behavior and the absence of growth factors will decrease the scaffold bioinductive properties.⁶ After the xenograft is decellularized it is then sterilized by exposure to radiation or ethylene oxide.

To date porcine SIS and BSMs have been strongly favored as potential donor xenogenic scaffolds for reconstructive urological surgery ([Appendix 4](#)). Both have had considerable clinical success as interposition urological grafts and form the basis of this review. Although the harvesting sites differ, both ECMs can support the growth and differentiation of multilayered urothelial cells while maintaining a connective tissue

layer that provides structural integrity to the bladder wall.⁷ SIS is harvested from the small intestine and BSMs originate from the bladder. The collagen components also differ to a small extent after decellularization and sterilization. The decellularized SIS scaffold is primarily composed of collagen type I with smaller amounts of collagen types III, IV, V and VI.⁴ In contrast, BSMs are rich in collagen types III, IV and VII.

BSMs possess an intact basement membrane which has many characteristics that favor its application to the genitourinary tract. An intact basement membrane can contribute to the prevention of urine infiltrating into deeper tissues which can in turn lead to graft shrinkage. Perhaps more importantly BSMs retain mechanical properties from original in vivo urological function that are closely matched to the targeted genitourinary organ.⁸

It has also been shown that significant limitations during the preparation process have hampered and delayed the progression of ECMs into the urological arena. Despite thorough decellularization and sterilization methods, xenogenic ECM materials still retain a significant portion of residual DNA and biosafety concerns exist.⁹ In addition, different preparation methods such as prehydration or lyophilization (freeze-drying) before implantation can affect the structural morphology, degradation rate and mechanical strength.¹⁰ These inconsistencies have inevitably led to a degree of apprehension among urologists when considering xenogenic ECMs for tissue engineering.

IMMUNE RESPONSE

Theoretically implanted ECM should not elicit an immediate or delayed immune response due to the xenograft acellular and avascular nature.¹¹ However, as elimination of all nuclear materials and cell membrane products is almost impossible during the decellularization process, a host derived immune response against the graft cell remnants may be expected. This hypothesis has been extensively studied by assessing the host cell mediated T-helper 1 (rejection) and T-helper 2 (accommodation) immune responses to implanted xenografts.¹²

Results from preliminary studies in mice are favorable, as the implanted SIS scaffold elicits an immune lymphocytic response that is predominately Th2-like.¹³ The Th-2 pathway stimulates the production of IL-4, IL-5, IL-6 and IL-10. These interleukins promote graft acceptance and prevent the activation of neighboring inflammatory macrophages.¹⁴ Activation of the Th2 pathway also promotes effective tissue remodeling, structural repair and functional recovery of the injured tissue after graft acceptance. Undoubtedly activation of this humoral response is encouraging, as

activation of the alternate lymphocytic pathway (ie Th1) produces an acute inflammatory reaction. Cytokines such as IL-2, interferon- γ and tumor necrosis factor- β activate neighboring macrophages and stimulate the differentiation of CD 8+ cells to a cytotoxic phenotype. This host derived inflammatory response ultimately leads to xenogenic graft rejection.¹⁵

The graft response to potential host derived pathogenic microorganisms has also raised concerns among urological surgeons as graft infection is associated with considerable morbidity. The xenograft response to Gram-positive and Gram-negative bacteria has been evaluated and compared to conventional synthetic graft materials (ie polytetrafluoroethylene) in animal studies.¹⁶ Interestingly xenogenic ECM materials were resistant to persistent bacterial infection after deliberate contamination at the graft implantation site.

This resistance has been attributed to the presence of multiple low molecular weight peptides that survive the decellularization and sterilization processes.¹⁷ These peptides demonstrate bacteriostatic activity against microorganisms and inhibit bacterial proliferation for up to 12 hours after initial exposure. Their antimicrobial activity protects the remodeling site from circulating pathogens.¹⁷ However, their origin and structural homology to natural antimicrobial peptides and defensins are important aspects that have not been clarified to date. Their spectrum of activity and pathways of incorporation are also poorly understood. These factors need to be thoroughly investigated so the extent of their antibacterial role can be clearly established.

REMODELING AND DEGRADATION

Biological growth factors in xenogenic SIS and BSM are key contributors to cell growth and tissue regeneration. Proteoglycans facilitate survival during matrix decellularization and sterilization by functioning as storage vessels.¹⁸ As the matrix is implanted, growth factors are released stimulating angiogenesis, host cell infiltration and mitogenesis ([Appendix 3](#)). Matrix degradation coincides with these events, and the degradation process is influenced by host derived enzymatic and cellular processes.⁸ During the degradation process growth factors dissociate from their binding proteins and are activated to promote tissue neovascularization. Matrix degradation and growth factor activation continue until the ECM scaffold is completely replaced by host cells.⁸

Common growth factors that influence tissue remodeling responses include vascular endothelial cell growth factor and basic fibroblast growth factor. Vascular endothelial cell growth factor has been shown to stimulate angiogenesis, vascular permeability, and en-

dothelial cell proliferation and migration, while basic fibroblast growth factor encourages wound healing.¹⁹ Other retained growth factors in the ECM include keratinocyte growth factor, which mediates epithelial cell proliferation and differentiation,²⁰ and platelet-derived growth factor- β polypeptide, which promotes chemotaxis, proliferation, angiogenesis and tissue remodeling. It appears that the strong remodeling effect exerted by biological growth factors is accentuated by cryptic peptides that are also released from the implanted scaffold during the degradation process. These peptides are involved in recruiting circulating bone marrow derived cells that can partake in long-term tissue remodeling processes.²¹

An ability to be completely degraded while stimulating a native remodeling response during a relatively short period is perhaps the most attractive feature of ECM. These impermanent properties were investigated by determining the rates of in vivo graft degradation and excretion in canine models during the 1990s.²² Studies show that xenogenic ECMs are rapidly degraded and absorbed when implanted in the genitourinary tract with up to 90% of the scaffold being replaced by host tissue within 28 days.²³ Generally excretion rates of all ECMs vary between 28 and 90 days depending on the type of tissue that is being remodelled.^{13,23} Naturally the process may be prolonged when multiple layers of xenogenic scaffold are implanted (eg 90 to 120 days).

Shortly after the degradation process the ECM briefly enters the blood stream and is excreted via the kidneys through glomerular filtration. This has been shown by measuring quantitative studies of ¹⁴C-labeled SIS after augmentation cystoplasty in canine models.²³ More than 50% of the scaffold was removed from the implantation site at 28 days and almost 100% of the scaffold was replaced by 100 days. During followup 95% of degradation products were found in the host urine.²³

MECHANICAL PROPERTIES

The xenograft remodeling capacity is dependent on the preservation of bioinductive growth factors during the sterilization process. Similarly its mechanical effectiveness is largely dependent on preserving intact collagenous arrangements and adhesive glycoproteins during this process. This is highlighted by the scaffold mechanical response to different sterilization techniques. Studies have shown that the graft uniaxial and biaxial mechanical properties are significantly reduced after exposure to gamma irradiation, electron beam irradiation and ethylene oxide.²⁴ The decrease in mechanical strength is dose dependent and higher doses can significantly affect graft durability. This important feature emphasizes the preparation difficul-

ties encountered between graft sterilization and constituent preservation techniques.²⁵

Mechanical limitations are further exacerbated during the initial remodeling response. Typically SIS and BSM show decreased mechanical strength after implantation caused by a temporal imbalance between the rates of scaffold degradation and infiltrating host cell deposition.²⁶ While the rapid degradation of implanted genitourinary ECMs is often lauded, one must consider the temporal mismatch that occurs between xenograft degradation and host derived matrix deposition. One study demonstrated a 30-fold decrease in bladder compliance (compared to the preoperative status) after canine bladder was replaced with SIS.²⁷ Importantly this imbalance between matrix degradation and deposition is temporary in nature, and a rapid remodeling response can occur once infiltrating host cells self-organize and begin producing their own ECM. However, patients may be subjected to complications such as poor compliance and deteriorating renal function until a gradual return to site appropriate mechanical strength occurs.²⁸

More recently the type of xenograft selected has been determined by the mechanical properties that are associated with the targeted anatomical structure or organ. This is because implanted ECMs retain defining features from their original in vivo function, suggesting that new host tissue is influenced by the 3-dimensional structure and fiber alignment of the donor matrix scaffold.⁸ From these findings one can argue that BSMs may be more mechanically suitable as a genitourinary tract applicant than SIS, because BSM scaffolds retain mechanical properties similar to genitourinary tissue, particularly the bladder.²⁹ After implantation BSMs preferentially align along the long axis of the recipient bladder and have a unique ability to stretch uniformly in all directions in response to genitourinary pressure and volume changes.⁴

In contrast, SIS regenerative potential in urological surgery is specifically dependent on the graft harvesting site from the small intestine. Distal porcine small intestinal segments demonstrate greater regenerative potential and significantly less graft contracture compared to proximal segments.³⁰ After surgical implantation SIS adopts an anisotropic collagenous fiber alignment with spirally arranged collagen fibers stretching and constricting along the preferred longitudinal axis in a manner similar to gastrointestinal peristalsis.³¹ A preoperative 3-dimensional understanding of the ECMs collagenous arrangement is necessary if mechanical properties of the recipient organ are to be maintained postoperatively.

GENITOURINARY TRACT APPLICATIONS

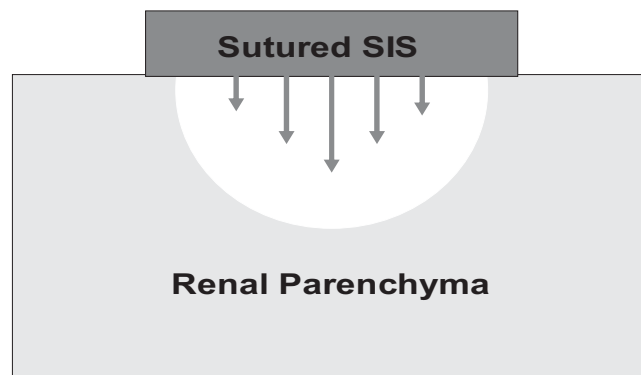
Repairing and reconstructing genitourinary tract structures with ECM scaffolds has been extensively

described with many successes and failures reported. An important urological advantage of xenogenic ECMs is their multifunctional potential after implantation due to their versatility. Acellular ECM scaffolds are useful for providing intraoperative hemostasis through compression and for repairing small tissue defects by releasing bioinductive growth factors. In addition, cell seeded and composite ECM scaffolds are capable of repairing larger and perhaps more complicated genitourinary tract defects and organs.^{32,33} Initially the majority of these studies were done in animal models. However, reports of successful human trials with encouraging short and long-term followup results are becoming more frequent.

Upper Urinary Tract

Kidney. NSS has gained widespread acceptance as an alternative surgical option for small renal masses. Previous studies have applied exogenous pro-coagulable materials like fibrin glue, Gore-Tex® or Teflon® to achieve sufficient intraoperative hemostasis during partial nephrectomy.³⁴ More recently the tensile strength and adaptability of prehydrated porcine SIS, applied in conjunction with pro-coagulable agents (eg Tachosil® or Tachocomb®), has been as effective in providing intraoperative and postoperative hemostasis after excising larger renal tumors.

Sufficient hemostasis is achieved through tension and downward compression exerted by the sutured SIS graft on the defect site as demonstrated in a study of 55 patients during a 3-year period (see figure).³⁵ Median tumor size was 4.5 cm (range 1.3 to 13) and median intraoperative blood loss was 730 cc (range 100 to 2,500), which is comparable to other conventional nephron sparing surgical methods.³⁶ A similar study described the role of SIS in ensuring a watertight closure of the collecting system and in minimizing parenchymal bleeding after NSS in 22 patients who underwent partial nephrectomy.³⁷ No postoperative parenchymal bleeds requiring transfusion or uri-



Xenogenic SIS exerts downward compression over renal defect to achieve hemostasis.

nary fistulas were reported during a mean followup of 18.4 months. These studies demonstrate that SIS is easily applied and highly effective at preventing postoperative complications. In this context the application of SIS merits further investigation, as the indications for NSS are continuing to expand.

Ureter. While xenogenic bioscaffolds are effective at providing intraoperative hemostasis in renal surgery, clinical limitations persist for ureteral reconstructive purposes. Previous experiments using synthetic materials like silicone-polytetrafluoroethylene and collagen tubes have failed because of infection, graft migration, crystal deposition and poor biocompatibility.³⁸ This is unfortunate because complicated strictures of the proximal ureter or long segment low grade urothelial tumors, which may be unsuitable for endoscopic ablation, can cause management dilemmas for urologists, particularly in the setting of a solitary kidney or complicated anatomy.

Early trials in pigs demonstrated initial success when allogenic SIS replaced a 7 cm left ureteral segment after two-thirds of the upper diameter was incised.³⁹ Vascularized segments of collagen and smooth muscle demonstrated angiogenic activity at autopsy 7 weeks later with no histological evidence of anastomotic strictures, stenosis, immunogenic reaction or graft rejection. A control was provided by performing a simple Davis ureterotomy on the opposite ureter with complete ureteral stenosis and no evidence of epithelial regeneration at 7-week followup. Similar findings were reported in another study that applied allogenic SIS to a 2 cm ureteral deficit encompassing half its circumference.⁴⁰ Autopsy at 9 weeks demonstrated ureteral patency with urothelial and muscular regeneration at the level of excision.

Unfortunately the validity of these encouraging results has been opposed by failures in other studies, with a study demonstrating 100% failure of tubularized acellular SIS segments to replace the ureter in 6 Yucatan mini pigs after 12-week followup.⁴¹ Theoretically these limitations may be addressed by preceding the xenograft with native cell types before implantation, as this method has been effective in maintaining patency after repairing larger (ie greater than 1 cm) urethral defects.³²

Bladder

The bladder can be affected by multiple pathological processes in which autogenous donor gastrointestinal tissue may be applicable for treatment purposes. However, the use of autogenous gastrointestinal segments is associated with complications that include metabolic abnormalities, urolithiasis and recurrent urinary tract infections.² These complications occur because the intestine is lined with absorptive mucus secreting

epithelium that is poorly compatible with a urinary reservoir.

Previously neobladders have been created with xenogenic biomaterials using cell based approaches in animal models.⁴² Studies in canine models have shown that a single layer of implanted SIS induces infiltrating mesenchymal cells to form 3 distinct histomorphological layers (ie urothelium, smooth muscle and serosa) when replacing a full thickness bladder defect.^{22,27} The remodeled SIS-ECM segment demonstrates contractile activity and functional innervation. However, significant limitations occur when these results are applied clinically. During followup the graft area can significantly decrease in size secondary to tissue fibrosis. Postoperatively this leads to complications such as decreased bladder compliance⁴³ and voiding difficulties.⁴¹

More recently composite scaffolds composed of cell seeded xenogenic ECMs and synthetic materials have appeared more suitable for replacing larger bladder defects, as neither scaffold possesses the mechanical strength required to maintain the structural integrity of larger organs when applied alone. This theory was investigated in a groundbreaking study by Atala et al in which native urothelial and smooth muscle cells were preseeded onto either BSM scaffolds or a composite scaffold of collagen and polyglycolic acid, which in turn were used as bladder augmentation segments in patients with neuropathic bladders due to myelomeningocele.³³ The scaffolds were implanted with or without an omental wrap in 7 pediatric patients with no postoperative complications after a mean followup of 46 months. Postoperative cystograms and urodynamic studies demonstrated that the greatest increase in bladder capacity and compliance occurred in the composite engineered bladders with an omental wrap (1.58-fold and 2.79-fold, respectively) while mean bladder leak point pressure at capacity decreased postoperatively by 56% (67 to 37.5 cm H₂O). However, additional prospective studies in human patients will be needed before widespread acceptance of these procedures.

Stress and Neuropathic Urinary Incontinence

Suburethral slings are the standard surgical treatment for urinary stress incontinence in females. Morbidity and increased operating time secondary to autogenous graft harvesting have encouraged the use of alternative graft materials such as allografts, xenografts and synthetic materials. Synthetic materials remain in situ and are not resorbed, but are associated with a 0.2% to 22% risk of local infection, erosion or extrusion.⁴⁴ SIS (Stratasis®) is commercially available for pubovaginal sling use in female patients with urinary stress incontinence. An initial study revealed promising results when 142 of 152 patients (93.4%) reported complete resolution of symptoms with no

postoperative complications after a mean followup of 27 months (range 4 to 48).

Worryingly inflammatory complications and concerns over graft durability have recently been reported. One study demonstrated a significantly inferior cure rate when acellular cross-linked porcine dermal slings were compared to rectus fascial slings stress after 3-year followup.⁴⁵ Another series revealed inflammatory complications in 31% (5 of 16) of patients who had a prehydrated SIS pubovaginal sling inserted.⁹ The etiology of the inflammatory reaction is unknown. However, the correlation between the high amount of residual porcine nuclei present and increasing incidence of postoperative inflammatory reactions has led to further skepticism of commercially available SIS among urologists.

Results appear more favorable when SIS is used to treat neuropathic urinary incontinence in the setting of neurogenic incontinence in the pediatric population. Autogenous rectus fascia is not always available because this patient population is more susceptible to poor nutritional status and more likely to have a history of abdominal surgery. Studies have shown that continence rates with SIS and autogenous slings are no different.⁴⁶

Of 20 patients treated with urethral slings synthesized from SIS 14 (70%) were fully continent after a 13-month followup (range 9 to 26).⁴⁷ Another study described successful use of SIS bladder neck slings in 21 pediatric patients with neuropathic urinary incontinence.⁴⁶ Continence was achieved in 75% of patients after a mean followup of 15 months (range 6 to 42). The sling is positioned posterior to the bladder neck after entering the endopelvic fascia and dissecting out the retropubic space. It is then crossed anteriorly, and tension sutures are applied and tied down over the rectus fascia. The encouraging results from these studies suggest that SIS may be a useful alternative when resection of the rectus fascia is not feasible. However, given the high long-term failure rates with porcine dermal fascial slings in adults, caution must be exercised in interpreting short-term results.⁴⁵

Urethra

Autogenous mucosa biopsied from the buccal cavity, colon, bladder and lingual area can be used to repair urethral deficits when limited urethral tissue is available for reconstructive procedures.⁴⁸ However, it is also recognized that complications such as infection, fistula formation, metabolic abnormalities, prolonged surgery and prolonged inpatient stay are associated with autogenous tissues.⁴⁹ The potential for reducing postoperative complication rates by applying alterna-

tive biomaterials has led to experimentation with xenogenic bioscaffolds.

Initially excellent patency rates were reported when BSMs were applied as an onlay graft to repair urethral deficits less than 0.5 cm in rabbits.⁵⁰ However, concerns were voiced when high failure rates secondary to stricture formation were reported when larger defects (ie greater than 1 cm) were repaired with tubular BSMs in 12 rabbits. This significant clinical limitation has been addressed by preseeding the BSM with allogenic cell types before surgical implantation. Recently BSMs that were preseeded with host derived urothelial cells, oral keratinocytes and epidermal cells were used to repair urethral defects greater than 1 cm in animal models. The preseeded BSM scaffolds demonstrated excellent patency rates. Maintenance of a nonobstructive outflow from the bladder while allowing tissue to regenerate across the urethral defect has been attributed to the porous nature of the BSM bioscaffold⁷ and to the remodeling effect that intrinsic growth factors exert on the seeded cell type.³² Urethral reconstruction with preseeded ECM scaffolds consistently demonstrates superior results compared to the ECM scaffold alone.³² However, further studies need to be done before this approach can be implemented clinically.

FUTURE PERSPECTIVES AND CONCLUSIONS

Significant experimental and clinical progress been made with tissue engineered xenografts in urological surgery during the last decade. Currently these bioscaffolds provide a useful alternative to effectively treat a number of urological conditions that affect the upper and lower genitourinary tract. Adaptable acellular scaffolds can provide hemostasis or induce tissue regeneration for smaller genitourinary defects. Conversely, cell seeded scaffolds can effectively replace larger genitourinary tract segments and composite cell seeded scaffolds can be used to successfully reconstruct genitourinary organs.

Importantly clinical concerns over residual porcine DNA after the preparation process and temporal mechanical limitations after sterilization techniques are important issues that need to be addressed to maintain the established clinical applicability of xenogenic bioscaffolds. If future studies can address and resolve these issues through the development of effective, standardized and repeatable ECM preparation techniques, xenogenic bioscaffolds may continue to transform the field of reconstructive urology.

APPENDIX 1

Current Applications of Xenogenic ECMs for Genitourinary Procedures

Organ/Tissue	Pathology	ECM	Urological Application
Kidney	Renal tumour	Acellular SIS	Haemostasis in nephron sparing surgery for renal tumors ^{35,37}
Ureter	Ureteral stricture	Acellular SIS	Ureteral replacement post excision of stricture or low grade TCC ^{39,*}
Bladder	Myelomeningocele	Cell seeded BSM + synthetic scaffold	Urinary bladder augmentation/reconstruction ³³
	Neuropathic urinary incontinence	Acellular SIS	Bladder neck slings ⁴⁶
Urethra	Stress urinary incontinence	Acellular SIS	Commercially available as a pubovaginal sling
	Stricture <0.5 cm	Acellular BSM	Onlay graft post urethroplasty ^{50,*}
	Stricture >0.5 cm	Tubularized cell seeded BSM	Maintains patency post reparation of larger urethral defects (ie >0.5 cm) ^{32,*}

* Procedure carried out on animal models.

APPENDIX 2

ECM Constituents and Their Specific Functions

Constituents	Features
Collagen	- Most abundant protein within ECMs⁴ - More than 20 different types have been identified - Provides distinct mechanical and physical properties to the ECM
Laminin	- Participates in cell-cell interactions through membrane-spanning receptor proteins known as integrins - Large adhesion glycoprotein - Involved in cell and tissue differentiation
Fibronectin	- Promotes tissue development and angiogenesis through beta-1 integrin chain - Extracellular glycoprotein - Promotes host biocompatibility
Glycosaminoglycans	- Induces cell adhesion by binding to the $\alpha_5\beta_1$ integrin - Mucopolysaccharides - Bind covalently to a protein core to form a proteoglycan molecule - Act as a reservoir when cells stop growth factor production
Growth factors	- Enable ECMs to store growth factors that may be used during tissue regeneration - Present in small quantities within ECMs - Naturally occurring substances capable of stimulating cellular growth, proliferation and differentiation

APPENDIX 3

Bioinductive Growth Factors in ECMs and Their Functions

Growth Factor	Function
Vascular Endothelial Growth Factor (VEGF)	Regulates angiogenesis by controlling blood vessel formation and growth ¹⁹
Platelet Derived Growth Factor (PDGF)	Deposition of granulation tissue and stimulation of angiogenesis ²¹
Bone Morphogenetic Protein (BMP)	Stimulates formation of bone and cartilage
Keratinocyte Growth Factor (KGF)	Epithelialization of wounds during healing ²⁰
Fibroblast Growth Factor (FGF)	Induces growth of fibroblasts and endothelial cells during wound healing ¹⁹
Transforming Growth Factor (TGF- β)	Reorganization of matrix molecules to improve dermal architecture and reduce scarring

APPENDIX 4

Differentiating SIS From BSM

Xenogenic Extracellular Matrix	Characteristics
Small Intestinal Submucosa (SIS)	- Derived from porcine jejunum - Primarily composed of the submucosal layer and the stratum compactum of the tunica mucosa⁴ - Mainly consists of type I collagen
Bladder Submucosal Matrix (BSM)	- Derived from the porcine bladder - Possesses an intact basement membrane consisting of collagen types IV and VII⁸ - Maintains genitourinary mechanical and functional features

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