Tissue-Engineering Bladder Augmentation

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45.1 Introduction

Tissue engineering (TE) and regenerative medicine combine cells and biomaterial techniques to encourage regeneration of new, healthy tissues and offer an alternative approach for the replacement of deficient organs. Currently, TE techniques have been developed to generate prostheses for different urologic tissues and organs [1]. The lower urinary tract (LUT) is responsible for urine storage and its evacuation. The urinary bladder and urethra consist of epithelium on the lumen surrounded by a collagen-rich connective tissue and muscle layer. Many pathologies affect the LUT and demand their replacement, and hence the health and quality of life of the patients at different ages and genders.

The main necessities for bladder surgical reconstruction are vesical exstrophy, neurogenic bladders, contracted bladder, and urothelial carcinoma. The gold standard technique for bladder replacement is the use of intestinal segments. Because the intestine is structurally and functionally different from the urinary bladder, many complications exist, such as hypocontractility, hematuria, dysuria, urolithiasis, neoplasia, ectopic mucus production, and metabolic imbalances due to urine absorption by the intestinal mucosa. Various urethral conditions, such as inflammatory and post-traumatic strictures, congenital defects, often require urethral reconstruction [2–6]. Currently, urethral conditions are treated with autologous grafts or flaps from genital skin or the buccal mucosa. There may be a limited donor supply of tissues needed for long segment replacement. Despite how good the initial result is, in the long term, all skin tubes (from genital or extragenital sources, whether used as grafts or flaps) have a tendency to deteriorate. For this reason, TE and regenerative medicine have evolved to compensate for the replacement of these organs to prevent complications and improve the quality of life for patients with major diseases necessitating bladder and urethral substitution.

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45.2 Cell Sources for Tissue Engineering of LUT

The ideal strategies for tissue engineering of LUT would be tissue-specific autologous cells harvested from an individual, cultured *ex vivo* to be expanded, and re-introduced into a second site for repair. Because urinary tract organs are composed of two cell types, a challenge would be to obtain differentiated smooth muscle and urothelial cells from progenitors or stem cells [7].

45.2.1 Progenitor Cells

These cells reside within each organ, have limited self-renewal capacity, and differentiate into only one defined cell type.

45.2.1.1 Epithelial Cells

(1) Autologous urothelial cells (UCs): classically, these cells are obtained from urinary bladder and have often been used in urethral and bladder reconstruction. (2) Autologous epidermal cells: these cells can be harvested from penile foreskin because of the abundant resources. (3) Autologous oral keratinocytes have also been used as a source of epithelial cells.

45.2.1.2 Smooth Muscle Cells (SMCs)

Autologous SMCs offer the potential for improved extracellular matrix (ECM) compliance and tissue elasticity, in addition to angiogenesis and epithelial maturation. In the bladder, SMCs are essential to allow for contraction of the engineered tissue for urine expulsion.

L. Liao, H. Madersbacher (eds.), *Neurourology*, https://doi.org/10.1007/978-94-017-7509-0_45

45.2.2 Stem Cells

Stem cells are undifferentiated cells that have self-renewal potential. *In vitro* studies have shown that human fat-derived mesenchymal stem cells (MSC) can increase smooth muscle gene expression. The combination of stimulation by humoral factors and co-culture with primary urothelial cells caused an increase of smooth muscle-specific gene expression in the treated MSCs. Bone marrow stem cells (BMSCs) can be differentiated into SMCs and UCs. Adipose-derived stem cells (ADSCs) have been successfully differentiated into UCs with all-trans-retinoic acid or co-culture with UCs [8].

45.3 Scaffolds for Tissue Engineering of LUT

The ideal biomaterial for bladder and urethral regeneration should have constant attachment of mature epithelial cell layer on the luminal surface and harbor multiple cell layers of SMCs on the outside, and should also provide adequate mechanical support and prevent collapse prematurely. The biomaterial scaffolds facilitate the delivery of cells to desired sites in the body, define the three-dimensional space for the formation of new tissues, and provide mechanical support for the regenerated tissues. Three different classes of biomaterials are used, as follows: naturally-derived materials, acellular tissue matrices and synthetic polymers. The self-assembly technique is an alternative method for tissue engineering [9].

45.3.1 Synthetic Polymers

Synthetic polymers, such as polyglycolic acid (PGA) and polylactic acid-co-glycolic acid (PLGA) are made of macromolecules assembled with covalent links. The advantage is the capacity to manufacture any form of an organ in three dimensions, quantitatively and reproducibly, and at a relatively low cost.

45.3.2 Acellular Tissue Matrices

Acellular tissue matrices are decellularized tissues, such as small intestinal submucosa (SIS) and bladder acellular matrix (BAM). They have the advantage of providing inherent bioactivity and mechanical similarity to native ECM due to the inherited presence of growth factors and ECM proteins.

45.3.3 Self-Assembled Engineered Tissue

The self-assembly method is able to produce a tissue built by the cells in which a dense ECM is completely produced by fibroblasts. The absence of an immunologic response should reduce the inflammatory and fibrotic reactions. Cells can receive the correct signaling for appropriate differentiation. Then, the transplanted engineered tissue is very similar to the tissue that has to be replaced.

Naturally-derived materials and decellularized tissue matrices have biological properties that better mimic native tissue or organ extracellular matrix, but these are limited in supply. In contrast, synthetic scaffolds can be produced on a large scale. The self-assembly technique proves to be useful for tissue reconstructions, ranging from skin-to-blood vessels [10].

45.4 Tissue Engineering of the Urothelium

TE of the urothelium can play a key role in reconstructive urology. Recently, graft tissues appear to have an advantage over matrices. These therapies depend on cell isolation and propagation *in vitro*. The choice of the correct cell source is crucial. The buccal mucosa was the most adequate substitute in urethral reconstruction [11]. TE has the potential to improve the quality of repair by identifying a new source of urothelium through the seeding of stem cells on an acellular tissue or scaffolds [7, 12, 13]. A functional multilayer urothelial sheath was recently cultivated from bladder wash-separated urothelium cells [14, 15].

45.5 Tissue Engineering of the Urinary Bladder

The generation of a bladder wall requires a multilayer cellular scaffold, and vascularization and innervation of the united smooth muscle structure. The addition of growth factors might enhance the regeneration of an acellular matrix [16].

A non-seeded scaffold technology can theoretically be the ideal strategy for bladder replacement because non-seeded scaffold technology is simple and does not require cell harvesting and *in vitro* culture. These scaffolds were thought to enhance tissue regeneration and recruit the local and systemic stem cells to the site of implantation to contribute to new tissue formation. When implanted, these scaffolds should imitate the natural ECM to orchestrate the different steps involved in the regeneration process, which is why naturally-derived ECM matrices were the first to be used for this approach. SIS and BAM were widely explored in experimental studies. For a long time now, it has been reported that acellular matrices are able to sustain proliferation of the urothelium and SMCs arising from adjacent normal tissue together with the blood vessel and nerve regeneration. Current developments in building BAM scaffolds facilitate the interactions between the matrix and surrounding tissue cells to allow the output of cell-seeded grafts used as the bladder replacement material.

SIS-based bladder tissue regenerative medicine allows the whole reconstruction of three normal bladder like-layers together with the vascular network [17, 18]; however, non-seeded scaffolds failed to show full regeneration of the bladder wall. Approximately 30% of the smooth muscle layer is able to grow back. The failure of cell-free scaffolds to replace bladder can be attributed to many factors, including extensive scarring within the graft due to xenographic or non-autologous nature of the graft and early exposure of the scaffold to urine, which induces scarring. Urine is toxic to the recruited progenitor and stem cells. Additionally, the lack of a muscle cell layer decreases the elasticity of the wall and prevents bladder contraction and cycling.

Atala et al. [19] reported a clinical trial of TE bladders. Bioartificial organs were created with autologous bladder cells seeded onto collagen-polyglycolic acid scaffolds and transplanted with an omental wrap in patients. The TE bladders displayed a physiologic tri-layered morphology and clinical parameters were stable over a 5-year period. The use of adult organ-specific cells has limitations, such as difficulty in harvesting, low proliferative capacity, and reduced functional quality. Seeding of scaffolds with stem cells might help to generate a bladder wall [20]. Synthetic polymers for cell-seeded 3D scaffold-based bladder tissue engineering, in addition to being endowed with essential biocompatibility properties, non-phlogogenic without inducing foreign-body tissue reactions, non-immunogenic, and non-cancerogenic must be able to adequately support seeded cell kinetic peculiarities, particularly due to interactions of specific soluble growth factors with transmembrane cell integrin receptors.

45.6 Clinical Application of Tissue-Engineering Bladder Augmentation

Bladder augmentation is required for some clinical indications in patients with NB. Due to the limited indications for auto-augmentation and ureterocystoplasty, enterocystoplasty remains the gold standard therapy [4]; however, enterocystoplasty is associated with serious complications. Composite cystoplasty has been proposed to avoid these complications by substituting materials. Our group and others reported promising results of SIS TE cystoplasty [17, 18]. SIS derived from pigs allows multilayered cell growth and urothelial differentiation, supports the growth of human urothelial cells, and is rapidly degraded. Our work showed that SIS caused bladder regeneration in rabbits (Fig. 45.1) [17]. Kropp et al. [21] also evaluated SIS as a possible AC material. Thus, SIS is the potential biodegradable material for clinical use in AC.

In recent years, bladder regeneration has been shown to be feasible using SIS TE techniques. SIS promotes regeneration of a variety of host tissues in clinical application. This

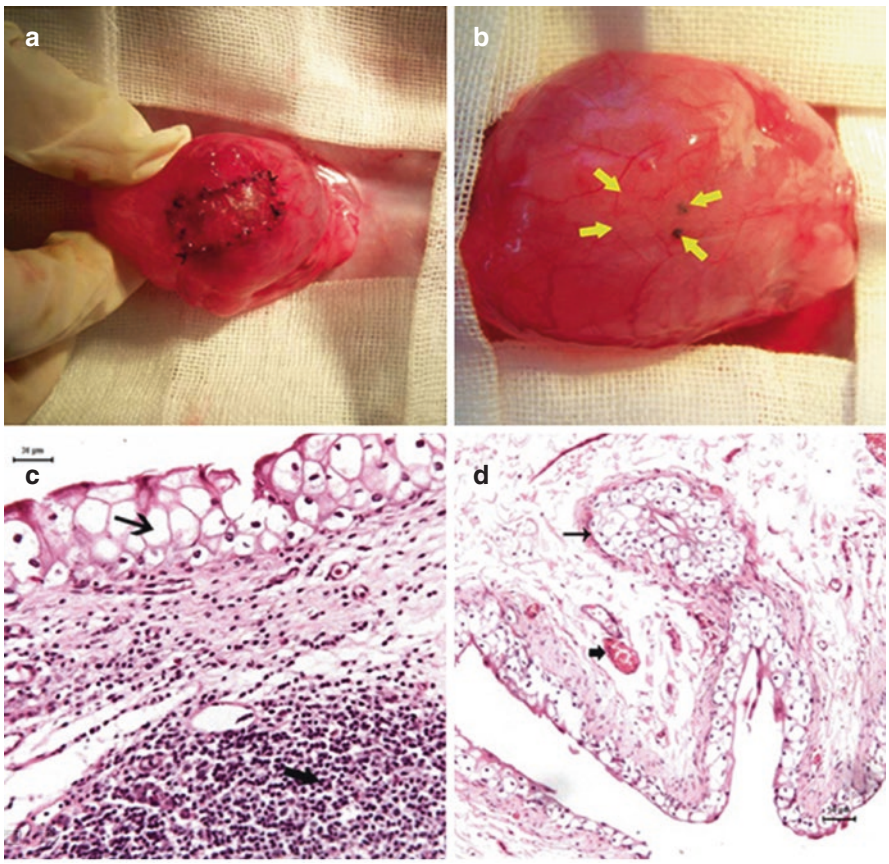

Fig. 45.1 Macroscopic and microscopic (HE) evaluation in the rabbit model. (**a**) The SIS patch (1.0 × 2.0 cm) was grafted onto the host bladder. (**b**) Regenerated tissue in the arrows 24 weeks post-operatively. (**c**) Thin arrow marked the regenerated transitional epithelium in the region of the SIS graft. Coarse arrow marked the infiltrated inflammatory cells (×20). (**d**) Thin arrow marked the regenerated transitional epithelium. Coarse arrow marked the new vessels (×10)

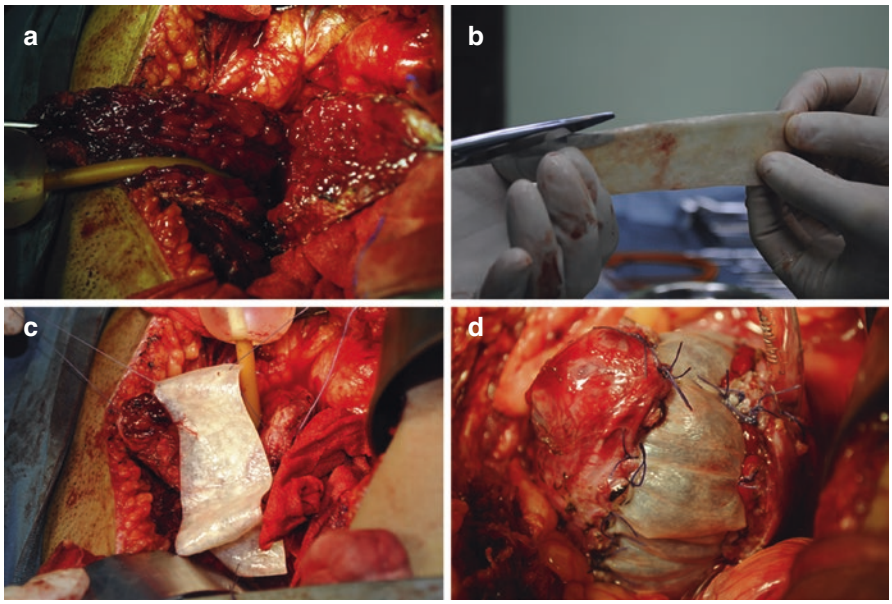

Fig. 45.2 Tissue-engineering bladder augmentation with SIS in a patient with neurogenic bladder. (**a**) Sagittal direction incision of the anterior and lateral walls of the opened bladder. (**b**) SIS scaffold fashioned into a strap shape. (**c**) SIS scaffold anastomosed to native bladder with running 3-0 polyglycolic sutures. (**d**) Cvovering SIS grafted bladder with soft perivesical tissue and omentum flap

characteristic is likely due to the persistence of many elements required for normal cell growth, differentiation, and functioning. We performed TE bladder augmentation with SIS in eight patients (A-H) with neurogenic bladder (Fig. 45.2) [18]. The approval for this study from the ethics committee of China Rehabilitation Research Center and the informed consent from participants were obtained.

Our study showed that SIS AC improved the functionality of bladders by decreasing the detrusor pressure, improving detrusor compliance, and avoiding renal deterioration. It has no gastrointestinal complications and metabolic abnormalities and enteric mucous. SIS-regenerated bladder had good compliance and capacity achievement, with contractile activity and radiologic and histological results. When large bladder tissue is in demand, acellular matrix grafting and cells are necessary. Biological material alone can facilitate tissue regeneration in partial AC. In our study, patient A completed 36 months of follow-up. The implanted bladder showed adequate capacity with preservation of renal function. This indicated that proper use of SIS during partial AC does not lead to fibroblast deposition, scarring, graft contracture, and a reduced reservoir volume over time (Fig. 45.3).

SIS has excellent host compatibility and remodeling function. Unlike enterocystoplasty, SIS AC is likely to expand gradually post-operatively. Thus, early bladder cycling by clamping the catheter intermittently is necessary for facilitating bladder remodeling. The anticholinergic agent is needed. Our study showed that MBC increased at the 1-month, and significantly at 3 and 12 months post-operatively, indicating that a strict regimen of post-operative CIC may also facilitate bladder remodeling. Urodynamics found that patients with reasonable bladder capacity tend to have good outcome. We reasoned that bladders with adequate basic capacity could be better expanded. Careful selection of patients with low-grade pre-operative UUT deterioration and watchful surveillance of post-operative bladder cycling are vital. The animal studies showed that non-seeded SIS could regenerate three layers of bladder tissues [22]. Other studies demonstrated the smooth muscle cell infiltration, vascularization and innervation in early stages, and did muscle bundle formation later. In our study, there was a smooth, epithelialized inner surface in the absence of the implanted materials 6 weeks post-operatively (Fig. 45.4).

Cystoscopy showed the surface of the grafted wall to be paler with a tendency to shed from the regenerative area 1 month post-operatively. It was difficult to distinguish the grafted wall from native bladder 6 months post-operatively. The complications of SIS AC are bladder stones and bladder rupture. No stone formation was observed in our study, possibly due to good urine drainage after surgery. The histological results partly support the theory of urothelial cell migration and structural properties of the original bladder wall may be transferred into the newly formed portion of the bladder. In our surgery, the SIS-grafted bladder was covered by perivesical tissue. Suprapubic catheter and perivesical drainage were all left *in situ*.

45.7 Summary

The work investigating TE patches using a variety of scaffolding materials continues to blossom, both in the laboratory and in the clinical setting. In bladder augmentation, SIS provides seemingly good results. These intermediate results are encouraged. The long-term follow-up to determine if these moderate-term good results are maintained is necessary. Whether or not non-seeded scaffolds are a better long-term source than cell seeded constructs remains to be seen, but at least at this juncture the technology with SIS is promising.

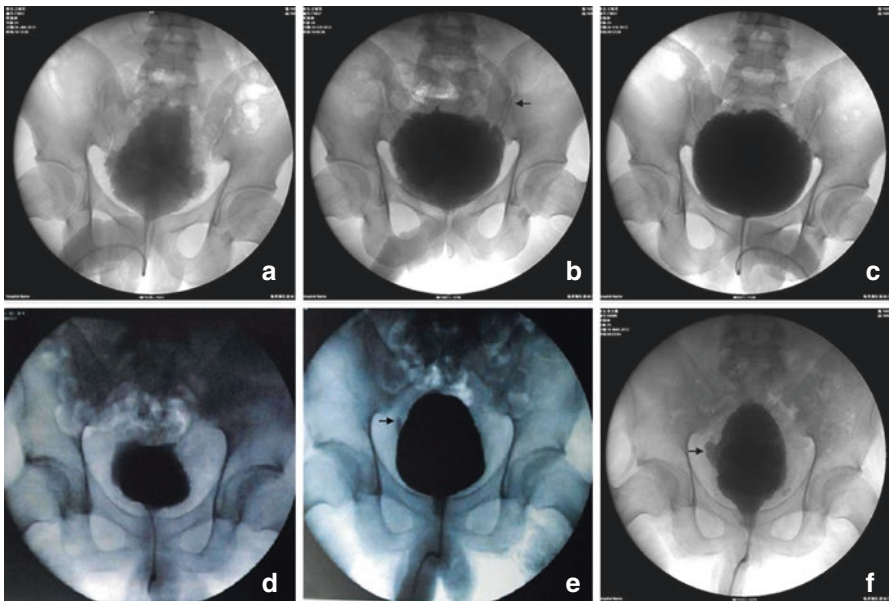

Fig. 45.3 Cystogram in patients with SIS bladder augmentation. (**a–c**) Cystogram in patient H. (**a**) Before surgery. (**b**) 1-month follow-up (dark arrow indicates vesical drainage). (**c**) 6-month follow-up. (**d–f**) Cystogram in patient A. (**d**) Before surgery. (**e**) 3-month follow-up (dark arrow indicates ureteral stump). (**f**) 36-month follow-up (dark arrow indicates ureteral stump). Ureteral stump was left at the time the ureter and bladder were disconnected when performing URI

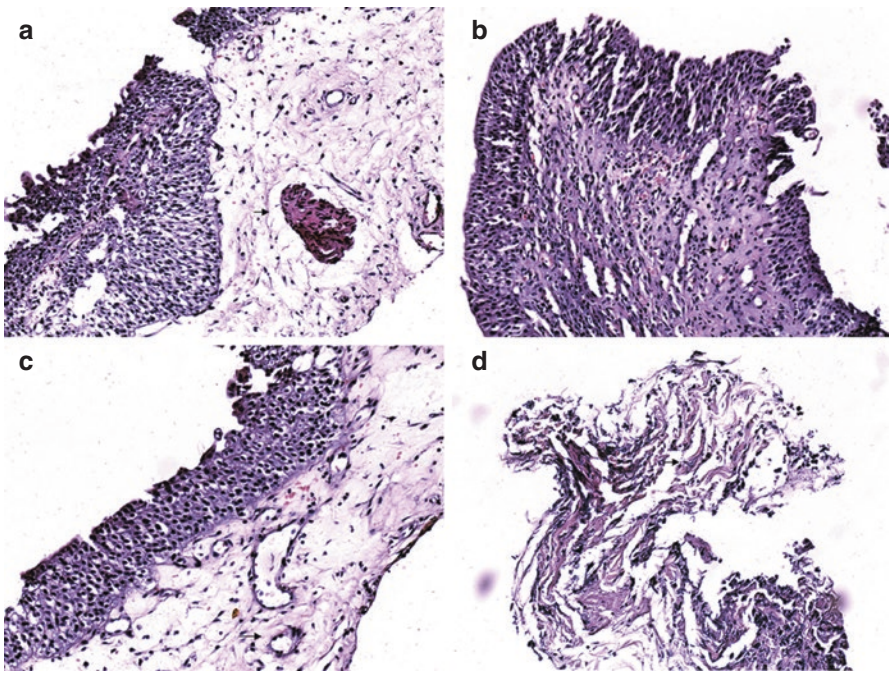

Fig. 45.4 Morphologic analysis of implanted engineered bladder in patient H. (H & E, reduced from 100). Cystoscopic biopsies of implanted engineered bladders 1–3 months post-operatively show extent of regeneration. (**a**) Inflammatory infiltration with follicular aggregation is present in lamina propria 1 month post-operatively. (**b**) Multilayered transitional epithelium fully covering regenerative bladder wall 6 weeks post-operatively. (**c**) New blood vessels proliferating into abundant connective tissue and fibroblasts 3 months post-operatively. (**d**) Connective tissue is regular without evidence of acellular scaffold 3 months post-operatively. Small amount of muscular fibers was observed

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