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Tissue Engineering in Low Urinary Tract Reconstruction

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1. Introduction

Acquired and congenital abnormalities of the lower urinary tract often require eventual reconstruction. Traditionally, different types of autologous tissue can be chosen for surgery, depending on which organ requires reconstruction. Bladder reconstruction, for example, is usually performed with intestinal tissue while urethral reconstruction can us buccal mucosa, lingual mucosa, colonic mucosa or prepuce skin. However, the problems of a shortage of patients' own tissues, and of nmany complications related to surgery, have not yet been resolved. There is therefore an effort to obtain sufficient tissue resources, to involve fewer complications, to reduce surgery to relatively minor invasion and to achieve better surgical outcomes. These goals may be attainable by the use of tissue engineering techniques.

Over the last 50 years, tissue engineering techniques for low urinary tract regeneration have been applied successfully in a variety of animal models and clinical patients. Rapid advancement has been made in this field, which has broadened the theoretical options for the future of low urinary tract reconstruction. These developments include improvements in cell culture techniques, such as the development of cell resources and identification of markers to isolate and characterize specific cell types. Many new types of natural and synthetic biomaterials for use as scaffold components have been created (1). In addition to these, the applications of nanotechnology and bioreactors have been strengthened within recent decades. Here, we review the literature on the basic principles and latest developments of tissue engineering technologies in lower urinary tract reconstruction.

2. Basic knowledge of tissue engineering in low urinary tract

2.1 Cell sources

2.1.1 Autologous stromal cells

Because epithelial cells are one of the most important components of the lower urinary tract, optimizing sources for them have always been a popular focus of investigators. Traditionally, urothelial cells obtained from bladder or urethra have often been used in previous studies (Fig 1a) (2,3). Although this technique exploits homotypy between the graft cells and host, it involves injury to the genitourinary tract and the operation is complicated.

Fu, *et al.* chose epidermal cells as graft cells because of its abundant resources; they can be obtained by a less invasive method than the traditional method of bladder or urethral biopsy followed by dissection of transitional cells. The results suggest that the epidermal cells can transform to transitional epithelial cells under the influence of the urethral or bladder environment (4). From our experience, we suggest using the oral keratinocytes, such as buccal keratinocytes and lingual keratinocytes, as a source of epithelial cells, (Fig 1b,c). Such cells express the β-defensin, IL-8, which can mediate an innate immune response against microbes (5). Therefore, compound grafts were easily resisted infection both in vitro and in vivo. In addition, these oral keratinocytes expressed AE1/AE3, which is similar to epidermal cells or urothelial cells in a previous report (6). However, 3T3 cells are usually needed as a feeder layer when culturing oral keratinocytes. The purification of oral keratinocytes therefore needs to be should be improved before clinical application.

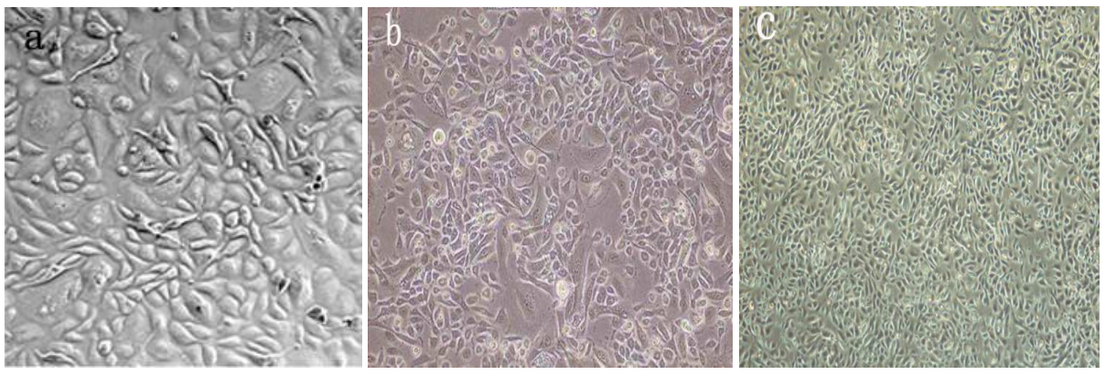

Fig. 1. Morphology the different kinds of epithelial seeding cells.
a. Bladder urethelial cells; b. buccal keratinocytes; c. lingual keratinocytes

To construct 3D bladder or urethral tissue, smooth muscle cell is also necessary. Previously, bladder smooth muscle cells were used for tissue engineering bladder reconstruction (Fig2a). The corpora cavernosa smooth muscle cells were used for constructing the corpora spongiosum, which is one of the most important components of the penile urethra (Fig2b). The advantage of using those cells is that some angiogenic growth factors and their receptors, such as Flk-1 and VEGF, are present in smooth muscle cells. They might contribute to the angiogenesis of bladder or urethral tissue (7). Since contamination by fibroblast cells is a

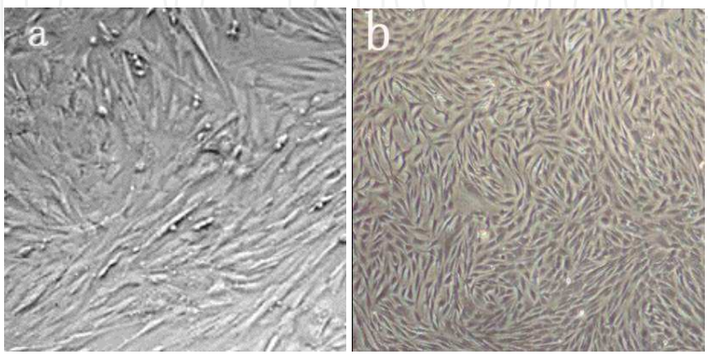

Fig. 2. Morphology the different kinds of smooth muscle cells.
a. Bladder smooth muscle cells; b. corpora cavernosa smooth muscle cells

problem during culturing of these, we advise that a velocity sedimentation method be used to evaluate the purification of smooth muscle cells. Of course, obvious trauma after the procedure is also the shortcoming of this method. As a result of these problems, smooth muscle cells are being replaced by muscle stem cells in urinary reconstruction.

2.1.2 Bone marrow and adipose derived stem cells

Stem cells from bone marrow (BMSC) have been characterized as being either hematopoietic or mesenchymal. They are easily isolated due to their affinity with and adherence to plastic dishes. Their ability to proliferate ensures that even a small number of BMSC multiply into millions of cells under the right culture conditions. Another merit of these cells is that they do not express MHC II, rendering them nonimmunogenic and thereby eliminating possible graft rejection (8). Previous studies showed that BMSCs contained higher concentrations of α-SM actin than did bladder SMC. Meanwhile, BMSCs showed strong response to the Ca^{2+}-ionophore, whereas fibroblasts did not contract their baseline even in the presence of calcium. Those results indicate that BMSCs and smooth muscle cells from low urinary tract are very similar (9). Therefore, BMSCs may serve as an alternative cell source in lower urinary tract tissue engineering.

Stem cells from adipose tissue (ADSCs) have also been popular in tissue engineering research. Adipose tissue is derived from embryonic mesodermal precursors and it contains multipotent progenitor cells that are capable of differentiating into mesenchymal tissue. Since adipose tissue contains 100-1,000 times more pluripotent cells per cubic centimeter

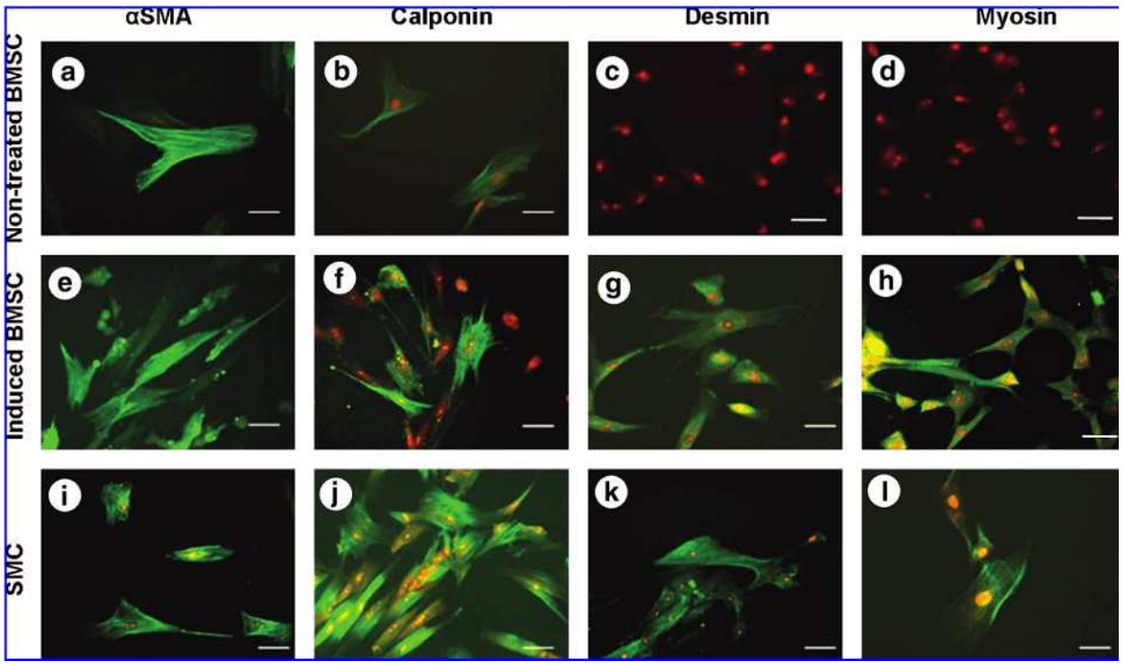

Fig. 3. Myogenic differentiation of human BMSCs using SMC-derived CM. Human BMSCs (p4) were stained with a-SMA (a, e, i), calponin (b, f, j), desmin (c, g, k), and myosin (d, h, l) antibodies without induction as negative control (a–d) and with induction for 14 days (e–h). SMCs were also stained with the same antibodies as a positive control (i–l). (Picture from ref 11)

than does bone marrow, it is easier to obtain ADCSs than other kinds of adult stem cells. Of course, immunoprivilege is also the advantage of this kind of cell. For these reasons, many investigators have selected ADSCs as an ideal source of seeding cells in lower urinary reconstruction, such as repair of bladder and urethra (10).

In many studies, mesenchymal cells have been found to differentiate into many different lineages, such as chondrocytes, osteoblasts, adipocytes, neurons and myoblasts. To urologists, the most interesting thing is the possibility for differentiation of BMSCs or ADSCs into smooth muscle cells and keratinocytes. According to previous reports, these stem cells can acquire a smooth muscle cell phenotype, staining positively for α-SMA, myosin and calponin after being cultured in conditioned medium. Also, culturing in the presence of other myogenic growth factors, such as PDFF-BB,HGF,TGF-β, can also lead to a phenotypic profile of smooth muscle cells (Fig 3) (8,11). Another group has demonstrated the differentiation of marked BMSC into urothelial cells on a seeded scaffold in porcine bladder augmentation, suggesting that mesenchymal stem cells can be made into urothelial cells. However, few additional reports support this result. Since the BMSCs and ADSCs are derived from the mesodermal lineage, more evidence is needed to support that ectodermal lineage cells can be induced from mesenchymal stem cells, such as BMSCs and ADSCs.

2.1.3 Other seeding cells

As well as the autologous stromal cells, BMSCs and ADSCs, other kinds of seeding cells have also shown possibilities for lower urinary tract reconstruction. Drewa (12) *et al.* used hair follicle stem cells for bladder regeneration in rats. This type of cell is CD34 positive, which facilitates the isolation of live epithelial cells with stem cell characterisitcs. In their study, Drewa *et al.* concluded that pluripotent stem cells within rodent hair follicle can differentiate into neurons, glia, keratinocytes and smooth muscle cells. They used an acellular matrix seeded with those cells and achieved a successful bladder wall reconstruction. Further research should be focused on better characterization of these cell populations and on the exact mechanism by which these cells enhance bladder regeneration.

Zhang's study focused on a subpopulation of cells isolated from naturally voided urine (13). This kind of cell demonstrated features typical of progenitor/stem cells, including expression of MSC and pericyte cell surface markers and clonogenic, multipotential, and plastic adhensive capacity. Furthermore, recent study showed that these cells have the capability to differentiate into the urothelial and smooth muscle cells (Fig 4)(14). The latest study has demonstrated the feasibility of forming a tissue-engineered conduit for use in urinary diversion by generating scaffolds seeded with human urine-derived stem cells.

Other cells, such as human amniotic fluid stem cells (AFS), human embryonic stem cells (ES) and human induced pluripotent stem cells (iPS), have also shown a potential for application in lower urinary reconstruction. However, most reports have been rather preliminary investigations. Several key points still need to be studied in depth before the cells can be used in patients.

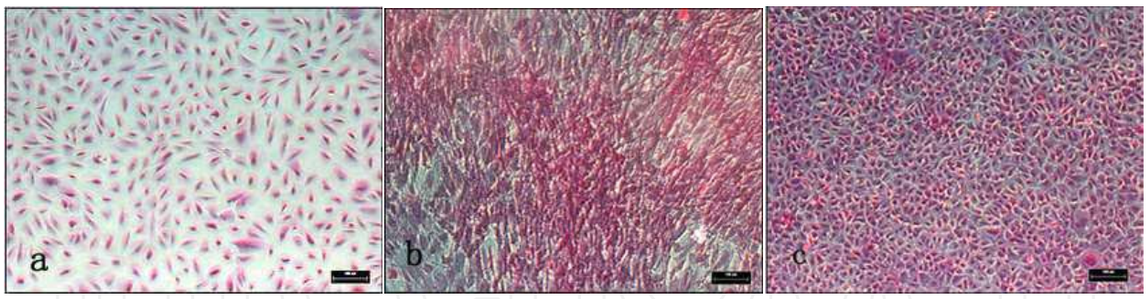

Fig. 4. Morphology of urine-derived stem cells obtained from upper urinary tract (USC-UUT) with differentiation. a. non-treated USC; b. The shape of -UUT changed from an oval to a spindle shape with the addition of myogenic medium; c. a cuboidal shape with the addition of uro-epithelial medium. Scale bar shown is 100 mm (Picture from ref 14).

2.2 Biomaterials

Creating an ideal biomaterial for lower urinary tract reconstruction has been an aspiration of urologists for over a century. An excellent biomaterial for tissue engineering should possess optimal mechanical properties, good biocompatibility, suitable three dimensional structures and degradation rates (15).

2.2.1 Traditional biomaterials

Traditionally, biomaterials can be classified into naturally derived materials, including chitosan, collagen; acellular matrix , such as small intestine submucosa (SIS), bladder acellular matrix (BAMG), acellular corpous spongious matrix (ACSM) and urethral extra matrix (UEM) , as well as synthetic materials, such as PGA and PLGA. Most of them have been used in animal models and human subjects, which will be discussed in the later section. Brehmer provides a useful classification of scaffolds into carrier-, fleece- and sponge-types, according to the structure of biomaterials (16) Carrier-type scaffolds are fiber meshes with very small pore sizes (<15 μm). The pore size of the sponge-type scaffolds is greater than 15 μm. Fleece-type scaffolds have huge interfilamentary spaces (200 μm). In our previous study, we compared the dimensional structures of SIS, BAMG, handmade PGA mesh and ACSM. SEM demonstrated that the pore size of the PGA (>200 μm) was the largest among all biomaterials. The surface pore sizes in SIS were significantly larger than BAMG (58.32 ± 10.31 μm vs 6.77 ± 0.49 μm; $P < 0.05$). Although a looser structure of BAMG could be seen with H&E staining, its pore sizes in surface views were smaller than those of ACSM (6.77 ± 0.49 μm vs 11.12 ± 1.43 μm; $P < 0.05$). An obvious difference of pore diameters in ACSM could be distinguished between urethral surface and cavernosal surface. (2.04 ± 0.32 μm vs 11.12± 1.43 μm; $P< 0.05$)(Fig 5)(17). This data can guide the following cell seeding procedure, since cellular growth and infiltration are strongly related to the scaffold's pore sizes. Of course, it should be noted that the structure of PGA or PLGA can be controlled now with the development of electrospin techniques. Therefore, the dimensional structure of synthetic materials is becoming more similar to the naturally derived scaffolds, and even to the original organs.

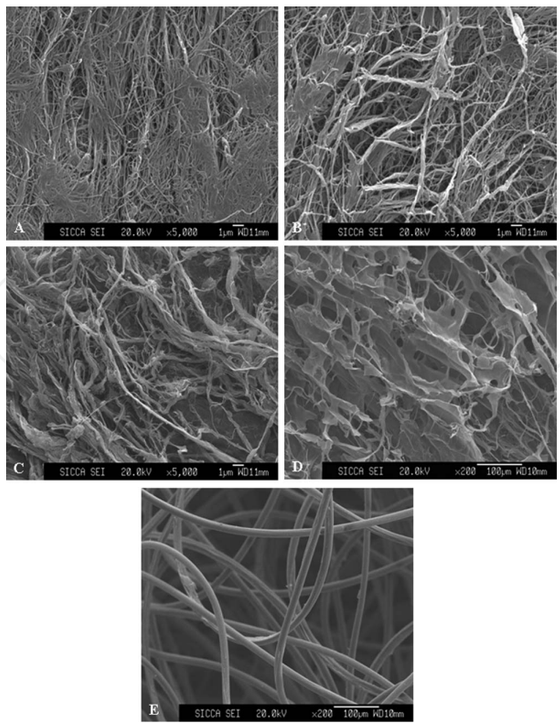

Fig. 5. EMS examination of different materials' surface. (A) urethral surface of ACSM, EMS×5,000, (B) cavernosal surface of ACSM,EMS×5,000 (C) surface of BAMG, EMS×5,000. (D) surface of SIS, EMS_200. (E) surface of PGA, EMS×200.

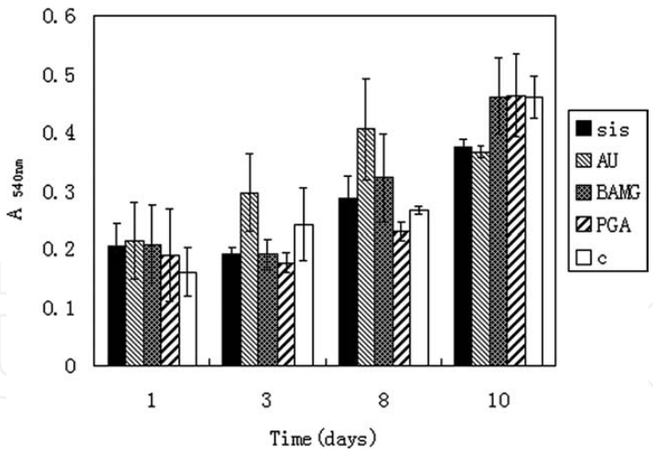

Fig. 6. Metabolic activity of CCSMCs cultured with extracts of various biomaterials or cultured directly in normal medium at 1, 3, 8, and 10 days, as determined by MTT assays. The difference between biomaterials and negative controls was not statistically significant

To address the issue of biocompatibility, our study used the MTT assay technique to evaluate cytotoxicity of different kinds of biomaterials. There were no statistically significant differences in MTT results between the cells cultured with biomaterial extracts and with controls (Fig 6). Thus, we may suggest that all scaffolds could be used safely for lower urinary tract reconstruction.

The mechanical properties of biomaterials are also key to successful reconstruction of the lower urinary tract. For urethral reconstruction, a uniaxial mechanical test is necessary to evaluate the scaffold. In our previous study, all biomaterials exhibited the classic biological nonlinear stress – strain response (Fig. 7) in a mechanical test. The ACSM showed good response in Young's modulus and breaking stress, these being better than in other scaffolds, even the normal rabbit urethra. For the bladder, physiological loading of the tissue involves compressive loads perpendicular to the bladder surface, induced by urine and surrounding pelvic tissues, so biaxial mechanical testing is more realistic. In addition, a burst experiment should also be considered (18) (Fig 8)

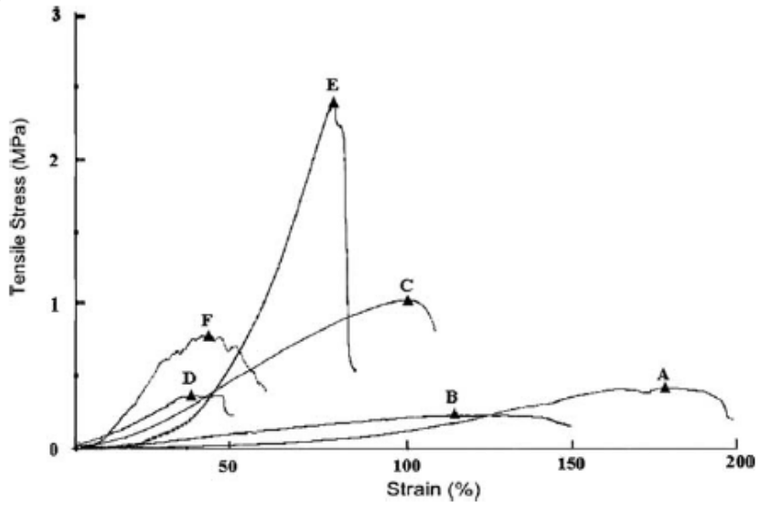

Fig. 7. Stress–Strain curves of various biomaterials. (A) Normal rabbit urethra; (B) SIS; (C) 4-layer SIS; (D) BAMG; (E) ACSM; (F) PGA

Fig. 8. Picture of ball-burst test with a ruptured test material. Arrow points at the rupture site from (ref 18)

2.2.2 Modified & advanced biomaterials

Since inherit weaknesses always exist in traditional biomaterials, many modified biomaterials have been studied to avoid them.

To enhance angiogenesis, some investigators have modified traditional matrices by incorporating heparin and subsequently loading the heparinized matrices with VEGF. Preliminary studies have shown that this loading of the matrices with VEGF increases the induction of microvessels in both heparinized and non-heparinized matrices, the effect being largest in the case of the heparinized matrices (19).

In order to control the three dimensional structure and degradation rate of synthetic scaffolds such as PGA or PLGA, electrospin techniques are often considered for tissue engineering in lower urinary tract reconstruction. Various materials have been examined for their ability to support cellular adhension, proliferation and formation of a multilayerd urothelium. The results provide the evidence that electrospinning scaffolds show significant benefitsa over commonly used acellular materials in vitro, and suggest that they should be further examined in vivo (20).

2.3 Advanced technique

2.3.1 Bioreactor

The bioreactor is a device that provides a fluid environment for the growth of cells for various applications, such as industrial fermentation and cell culturing. Bioreactors should be introduced in tissue engineering to optimize, through fluid shear, oxygenation and the supply of nutrients, the growth of cells on a 3D scaffold. This approach has been shown to result in better tissue-like constructs than do conventional static culture conditions (21). It is possible to use in vivo graft sites as 'bioreactors' that feature flowing fluids (blood). An example that is commonly used in tissue engineering for lower urinary reconstruction is the greater omentum. Baumert et al (21) used urothelial and smooth muscle cells to seed a sphere-shaped small intestinal submucosa matrix, which was transferred into the omentum after 3wk of cell growth. By this approach, they obtained tissue engineered bladder with a wall thickness was 4 mm. The construct presented a multilayer urothelium on the lumial aspect and deeper fascicles of organized tissue composed of differentiated smooth muscle cells and mature fibroblasts. There was no evidence of inflammation or necrosis (Fig 9). Gu et al (22) implanted 8Fr silastic tubes into the peritoneal cavity of a rabbit. Those tubes were harvested and the tubular tissue covering the tubes was reverted. A pendulous urethral segment of 1.5 cm long was totally excised and urethroplasty was performed with the reverted tubular tissue in an end-to-end fashion. Finally, the results of study showed that the recipients' peritoneal cavity can be used as bioreactor for tissue engineering urethral reconstruction.

More manufactured bioreactors have been designed for tissue engineering bladder. In order to mimic the dynamics of the urinary bladder, bioreactors that imitate the filling and emptying of a normal bladder have been suggested. A bladder bioreactor built this way should be able to recapitulate those dynamics while providing a cellular environment that facilitates cell-cell and cell-matrix interactions. Under the mechanical stimulation from bioreactor, the physiological and mechanical properties of the bladder can be improved. The growth behavior of urothelial cells and bladder smooth cells can be changed, resulting in the cells undergoing adaptive changes in mechanically-stimulated environment (23, 24).

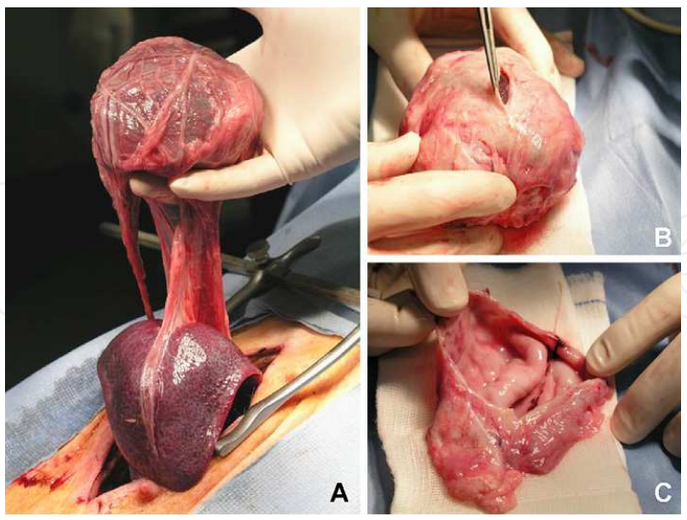

Fig. 9. Harvesting of the matured construct 3 wk after implantation in the omentum (ref 21)

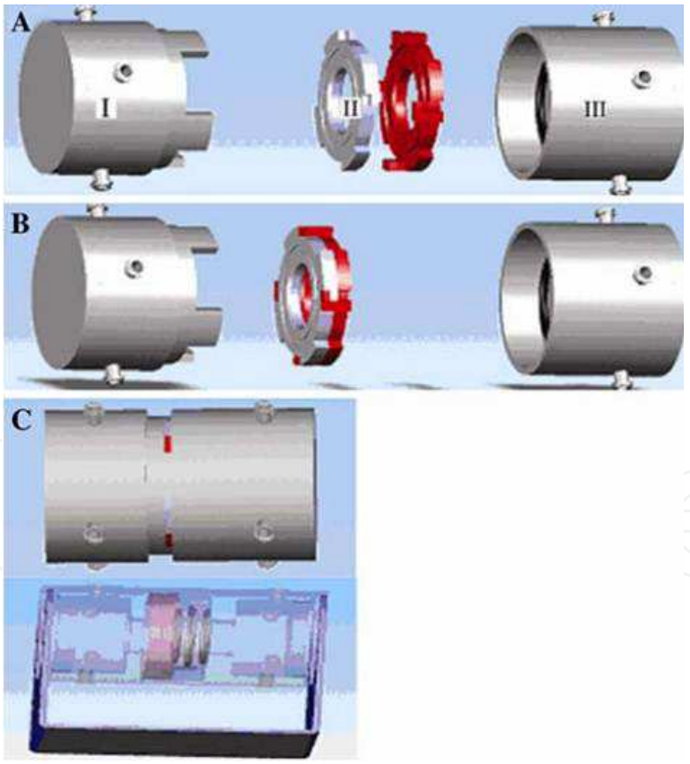

Fig. 10. a. Disassembled urinary bladder bioreactor. I. This chamber will be subjected to controlled pressure and hence would mimic in vitro the urinary bladder chamber. II. Tissue engineered construct ring. III. Compliance chamber (cell culture medium will be re-circulated to accommodate the expansion of the scaffold upon pressure generation).
b Interlocking discs for cell-seeded scaffold. c Assembled bioreactor (ref 23)

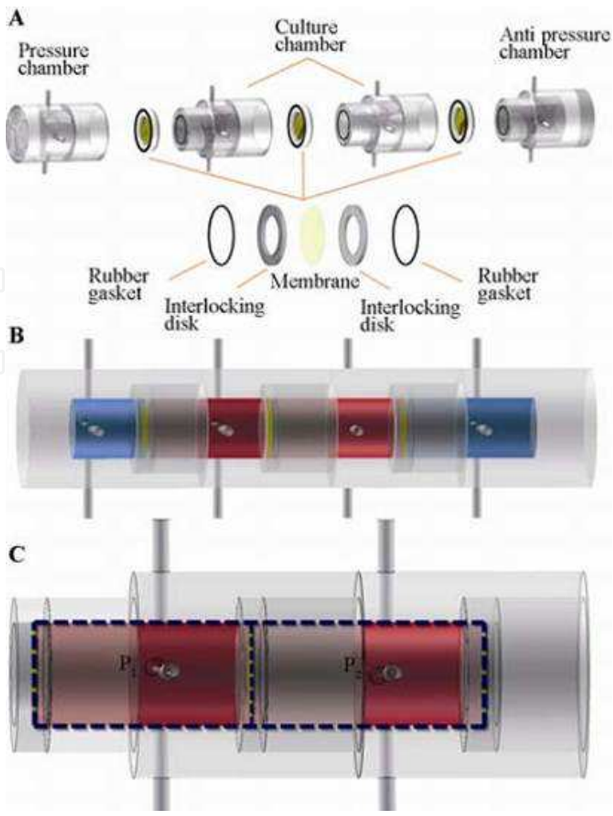

Fig. 11. The bioreactor system. A: A diagram of the disassembled bioreactor, showing the 2 pressure chambers and 2 culture chambers, separated by 3 interlocking rings with elastic membrane. Every interlocking ring was 2 interlocking disks that hold the cell-seeded membrane, which was glued by α-cyanoacrylate. B: The assembled bioreactor with the ports, to which tubing would be attached for medium flow and pressure monitoring. The red parts are culture chambers, blue parts are pressure chambers. C: The assembled culture chambers, the dashed frame showing the pressure P1 and P2 on both sides of cell-seeded membrane. the elastic membrane deformation was driven by pressure difference (P1-P2). (ref 24)

2.3.2 Nanotechnology

Nanotechnology has largely emerged in the last decade of the 20th century as a potential new enabling technology for medicine. For bladder reconstruction, this technology provides a new set of tools to solve many problems that may encountered during the reconstructive procedure. Especially, the incorporation of nanotechnology into bladder tissue engineering materials provides for better bladder materials. Recent published work has demonstrated that increasing of material surface roughness at the nanoscale can improve the adsorption of select proteins important for bladder cell functions (25). Furthermore, some reports showed more bladder smooth muscle cell attachment and growth on polystyrene nanofiber scaffolds fabricated, using an electrospinning technique, to possess surface features at the nanoscale. Cellular adhesive and proliferative ability of keratinocytes were also improved in the nanoscaled scaffold (26).(Table1)

As well as the electrospinning technique mentioned above, another useful nanotechnique is the use of nanoparticles as a delivery system. Mondalek et al (27) investigated the use of PLGA nanoparticles to alter the permeability of SIS scaffolds. Preliminary results indicated that particles ranging from 200 to 500 nm would become imbedded in the SIS scaffold. Particles below this size range would pass through the graft and not become entrapped, and particles above this size range could not penetrate the scaffold. Those results provided the possibility of using nanoparticles to deliver growth factors into seeded cells and scaffolds, to enhance the regeneration of lower urinary tract.

	Bladder Cell Adhesion	Bladder Cell Proliferation	Bladder Cell Synthesis of Proteins	Other Important Functions
Nanostructured PLGA	Highly increased (SMC)[27]	Increased (SMC)[45]	Increased synthesis of elastin and collagen[46]	Decreased formation of calcium stones[29]
	Highly increased (UC)[29]	Highly increased (UC)[29]		
Nanostructured PU	Increased (SMC)[27]	Small increased (SMC)[45]		Decreased formation of calcium stones[29]
	Increased (UC)[29]	Increased (UC)[29]		
Nanostructured PCL	Highly increased (SMC)[27]	Increased (SMC)[45]		
Polymer nanofibers		Increased (SMC)[51]	Production of collagen[51]	Increased cell migration[51]

JC, urothelial bladder cells; SMC, smooth muscle bladder cells.

Table 1. Nanotechnology Approaches to Increase Bladder Cell Functions (ref 26)

2.3.3 Oxygen generating scaffolds

The limitation of oxygen diffusion has led to the general concept that cell or tissue components may not be implanted in large volumes. Many efforts have been made to overcome this limitation. Recently, implantable oxygen releasing biomaterials have been developed in order to provide a sustained release of oxygen to cells and tissues with the goal of prolonging tissue survival and decreasing necrosis (28). In those studies, an oxygen rich compound of sodium percarbonate or calcium peroxide was incorporated into films or 3D constructs of PLGA and used for in situ production of oxygen. In vitro, release of oxygen could be observed from the film more than 24h. Furthermore, these biomaterials were able to extend cell viability growth under hypoxic conditions. Those findings indicate that the use of oxygen generating biomaterials may enhance the scaffold neovascularization after implantation (29). All results suggested that oxygen generating scaffolds can be used for lower urinary tract reconstruction in the future.

3. Applications of tissue engineering in the lower urinary tract

3.1 Bladder reconstruction

Congenital disorders, cancer or trauma can lead to obvious bladder damage. For patients with these problems, bladder reconstructive procedures may be considered. Although gastrointestinal segments are commonly used for bladder augmentation or replacement, multiple complications cannot yet be completely avoided; they include infection, metabolic disturbance and ureolithiasis. A number of animal studies and even clinical experiences have, however, shown the possibility of using tissue engineering techniques to reconstruct bladder tissue. In the laboratory, tissue could be engineered to have function equivalent to

the original tissue. In the clinic, patients provided with engineered bladder tissue have obtained satisfactory results.

3.1.1 Animal experiments

Since 1955, many investigators have tried to use different kinds of scaffold for bladder reconstruction in animal models; these have included polyvinyl sponges, polyethylene moulds, Teflon, gelatin sponges, and decellularized pericardial tissue. The outcomes in most studies were unsatisfactory (30). One really successful experiment was reported by Kropp BP et al. (31) in 1995. In this study, the rat underwent partial cystectomy with immediate bladder augmentation with SIS. Host cellular infiltration into the scaffold could be seen 2 weeks after operation. By the end of 48 weeks, the SIS graft presented the three-layered structure of normal bladder, which was indistinguishable from the original bladder. This preliminary study demonstrated the feasibility of using an optimal tissue engineering scaffold for bladder reconstruction. Further study has shown that muscarinic, purinergic and functional cholinergic innervation occurred in rats (32). More recently, bladder regeneration has shown to be more reliable when the SIS was derived from the distal ileum (33). However, graft contraction could be observed in large animal models after using SIS for bladder augmentation, which means that pre-seeding cells may be necessary for tissue engineering-based bladder reconstruction in humans.

Zhang et al. first seeded human bladder urothelial cells and smooth muscle cells onto the SIS by a sandwich culture method (34). This kind of seeding method resulted in organized cell sorting, formation of a well-defined pseudostratified urothelium and multilayered smooth muscle cells with enhanced matrix penetration (Fig 12). The initial study demonstrated that using SIS combined with cell culture could be a valuable model for the study of tissue engineering in bladder reconstruction. To address the problem of graft contraction, Brown et al. showed that opposite-side co-culture of smooth muscle cells and epithelial cells produced a less pronounced matrix contraction than same-side co-culture (20) (Fig 13). The other problem to be addressed in tissue engineering bladder in vitro is cellular infiltration. Recently, Liu et al used preacetic acid (PAA) and Triton X-100

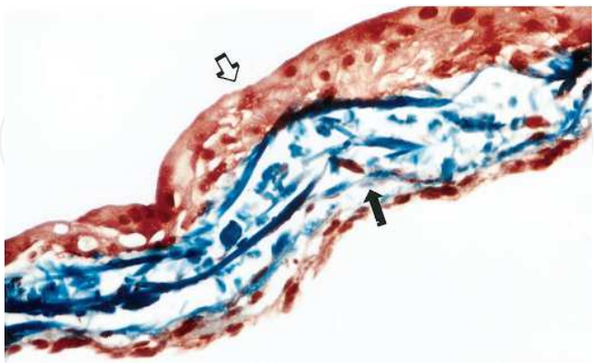

Fig. 12. Sandwich coculture at 28 days shows similar growth pattern to layered coculture technique except that urothelial cells and smooth muscle cells are on opposite sides of small intestinal submucosa membrane. Pseudostratified layer of urothelium is on mucosal surface (open arrow) while multiple layers of smooth muscle cells are on serosal surface and are penetrating into matrix of small intestinal submucosa membrane (solid arrow)(ref 34)

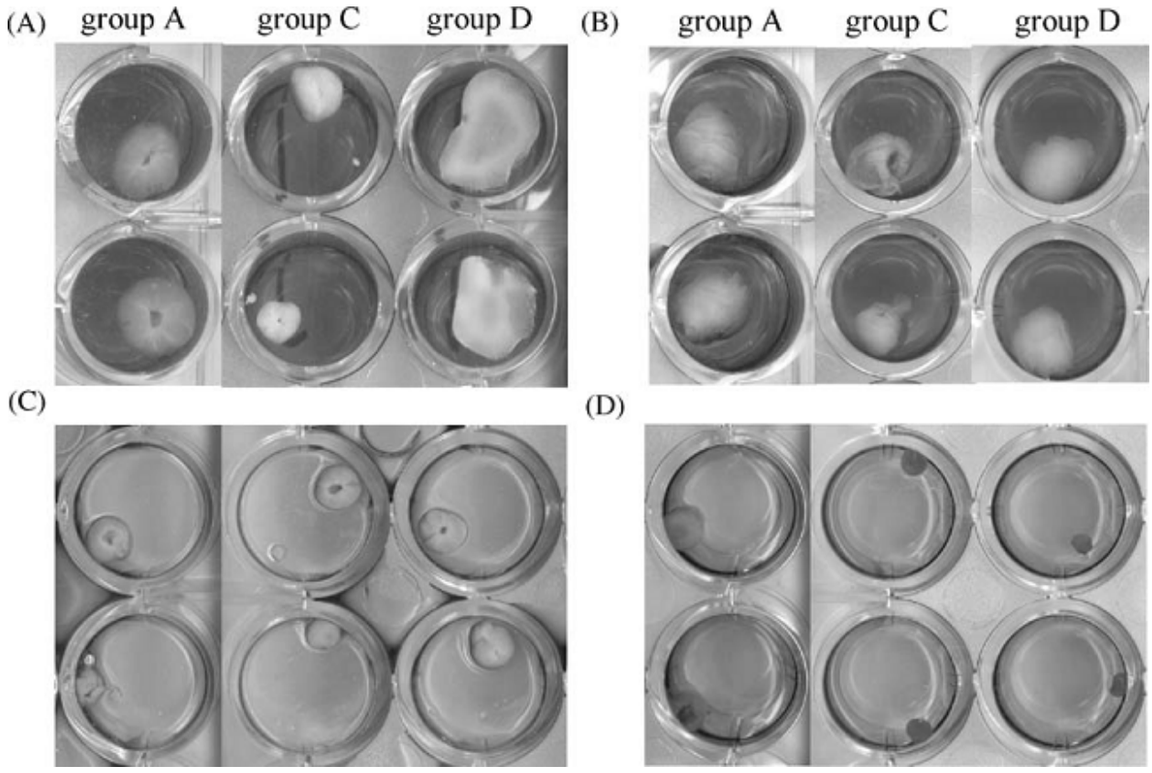

Fig. 13. Gross appearance of cell seeded constructs at 7 and 28 days post seeding. (A) Collagen Gels, 7 days. (B) BAM, 7days. (C) Collagen Gels, 28 days. (D) BAM, 28 days. Group A: SMCs only. Group C: same side co-culture. Group D: opposite side co-culture (ref 20)

to treat the acelluar matrix for bladder tissue engineering reconstruction in vitro (35). This method led to high porosity on the surface of the matrix with about 75% of normal strength. After 3-D dynamic culture, cells could penetrate deeper into the lamina propria of the matrix compared to untreated matrix. (Fig 14). The authors believe that treated scaffold might be more suitable for bladder tissue engineering reconstruction. In order to enhance the vascularization of tissue engineered bladder scaffold in vitro, Baumert et al. transferred the compound matrix into the greater omentum, which has been mentioned above (21).

After meticulous investigation in vitro, Yoo, et al. used scaffold seeded with multiple cell types to reconstruct bladder tissue in 10 beagle dogs, which on which a partial cystectomy had been performed. As a result, 99% a increased in capacity was achieved in the reconstructed bladder. Immunocytochemical analyses confirmed the urothelial and muscle cell phenotypes and showed the presence of nerve fibers (36). Compared to the the technique of seeding with stromal cells, mesenchymal stem cell-seeded scaffold is becoming much more popular in bladder reconstruction. Chung et al. first performed bladder reconstruction using a BMSCs-seeded SIS in rats. At the level of gene expression, regenerated bladder was similar to the control bladder (37). Compared with the BMSCs, ADSCs can be procured more easily. Therefore, we chose ADSCs-seeded scaffolds for bladder reconstruction. At the end of 24 weeks after the operation, the reconstructed bladders reached a mean volume of 94.68±3.31% of the pre-cystectomy bladder capacity in our study. Smooth muscle cells, urothelium and nerve bundles could be detected by

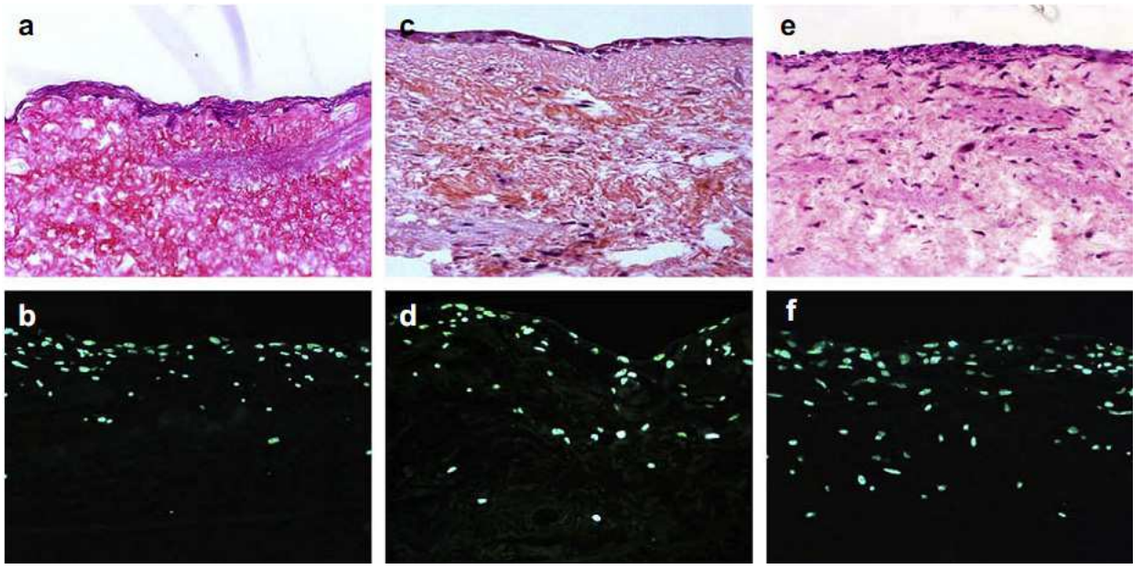

Fig. 14. Cell penetration in 5% PAA-treated BSM in different speeds (0, 10 and 40 rpm) in three-dimensional dynamic culture. Smooth muscle cells and urothelial cell were seeded as layers and co-cultured on the submucosa side of 5% PAA-treated BSM using static culture (left column; a,b) and 3-D rotation culture conditions at 10 (middle column; c,d) and 40 rpm (right column; e,f). H&E staining (a,c,e) and DAPI staining (b,d,f) are shown at 200. Compared with static culture (left column), the cells grew uniformly with deeper penetration in the matrix using 3-D dynamic culture (middle and right column). The cells grown at 40 rpm had deeper penetration of cells within the matrix (e,f) compared to cells cultured at 10 rpm rotation speed (c,d) (ref 35)

immunohistochemical assays. On the contrary, the mean bladder volume was 69.33±5.05% in the control group, made using unseeded scaffolds, and in these there was no evidence of organized muscle or nerve tissue (10) (Fig 15,16). In our study, we also noted that the optimal area for bladder regeneration using seeded scaffold is more than 40-60%, since smaller areas can be regenerated by native bladder tissue. These data provide a useful reference for further clinical application. Other stem cell-seeded scaffolds have also been reported for bladder reconstruction, such as hair-follicle stem cell-seeded scaffolds and urine-derived stem cell-seeded scaffolds (12). However, the number of reports is limited and the actual effectiveness of those scaffold need to be further studied.

As well as traditional cell seeded scaffolds, many modification techniques have been used for tissue engineered bladder reconstructions in animal models. Gregory et al. seeded human adipose stem cells onto PLGA (85:15) bladder dome composites and grafted the result into rat hosts. Results showed that bladder capacity and compliance were maintained in the cell-seeded group throughout the 12 weeks (38) (Fig 17). SIS, modified by hyaluronic acid nanoparticles, has been used for bladder reconstruction. Urinary bladder augmentation has been performed in beagle dogs following hemi-cystectomy using nanoparticle-modified SIS. The results showed that the modified scaffold had significantly higher vascularity compared to unmodified one. This report demonstrated that the nanotechnology can represent a new approach for modifying biomaterials in bladder reconstruction (39). Wei et al.

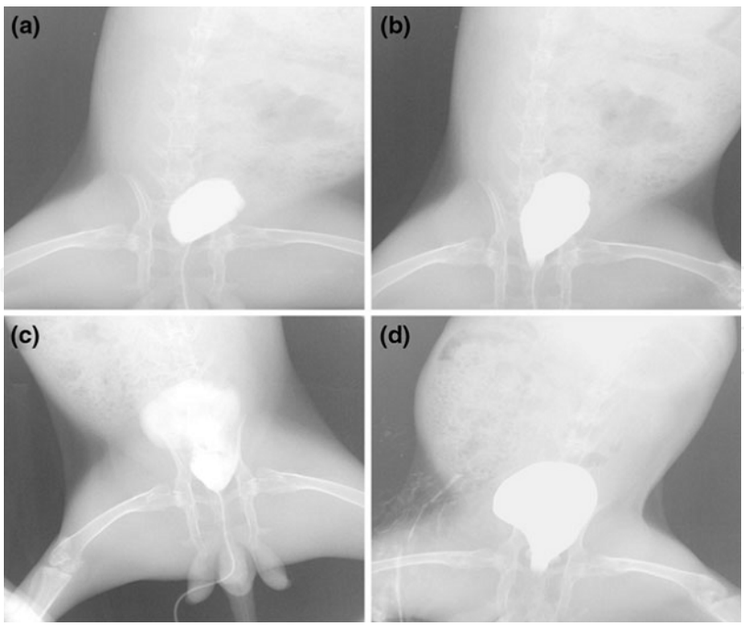

Fig. 15. Cystographies of bladders reconstructed 4 weeks postoperatively. a The control group, b the experimental group 24 weeks postoperatively, c the experimental group and d the experimental group. Cystography demonstrated an improvement in both the shape and capacity of bladders reconstructed with seeded matrices

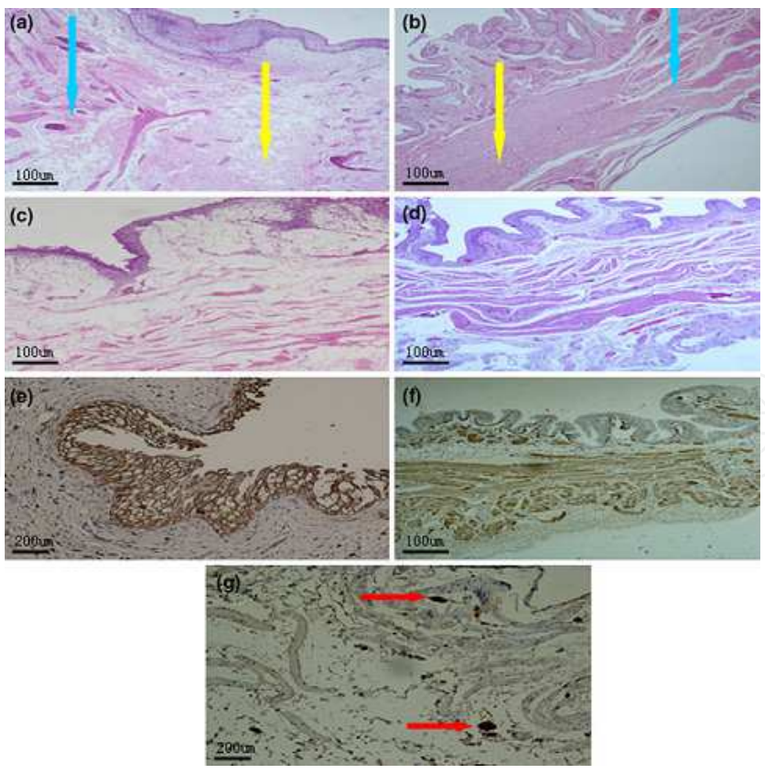

Fig. 16. Histological features of the transplanted grafts. Four weeks postoperatively, native bladder tissue (blue arrow) and in the graft (yellow arrow). a The control group, b the

experimental group 24 weeks postoperatively, c in the control group there is no evidence of organized bladder tissue regeneration, d in the experimental group, the grafts had formed a multilayer epithelium with organized smooth muscle cells. Immunohistochemistry of the transplanted grafts. e Staining with cytokeratin AE1/AE3. α-SM actin. g S-100 (arrows).

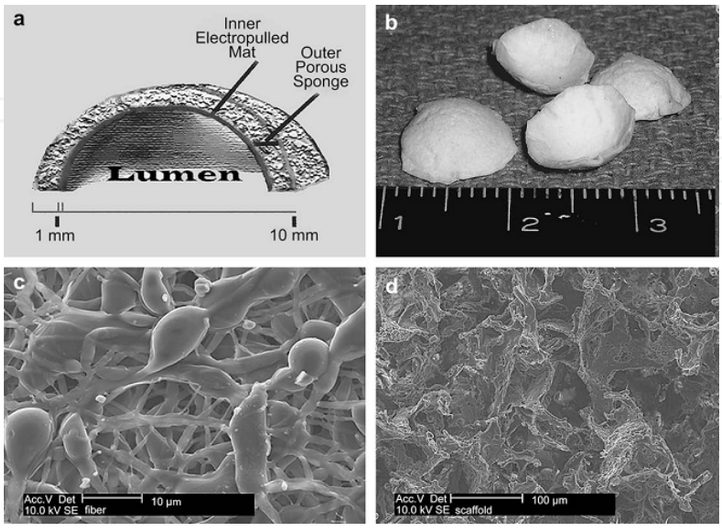

Fig. 17. Construction of the three dimensional synthetic bladder composite. a: Schematic and b: gross micrograph of the three dimensional bladder composite. c:PLGA electropulled microfibers comprising the luminal layer. d: PLGA porous sponge was used as the outer layer (ref 38).

designed a bioreactor to simulate the mechanical properties of bladder. This system successfully generated appropriate pressure waveforms. The viability of cells and tissue structures observed after culture in simulated conditions showed that mechanical stimulation improved the arrangement of cells on scaffold (24).

3.1.2 Clinical application

Although the reports about bladder reconstruction using tissue engineering techniques are few, they are the landmarks in tissue engineered lower urinary tract reconstruction. In Atala's famous study, seven patients with myelomeningocele with high-pressure or poorly compliant bladders enrolled. Urothelial and muscle cells were seeded on a biodegradable bladder-shaped scaffold made of collagen and PGA. Then the biomaterial was used for reconstruction with an omental wrap (40). (Fig 18) After the operation, none of the ultrasounds showed any abnormalities. The cystogram showed the regular shape of bladder after the reconstruction. Urodynamic studies demonstrated significant improvement in volume and compliance in the composite engineered bladders (Fig 19). Postoperatively, it is difficult to distinguish the margin between the composite matrix and the native bladders grossly. All biopsies showed a trilayered structure, consisting of a urothelial cell-lined lumen surrounded by submucosa and muscle. During the post-operative follow-up period all patients had a stable renal function in which serum creatinine was similar to the preoperative status. No metabolic abnormalities were noted. There was no evidence of urinary calculi during the study.

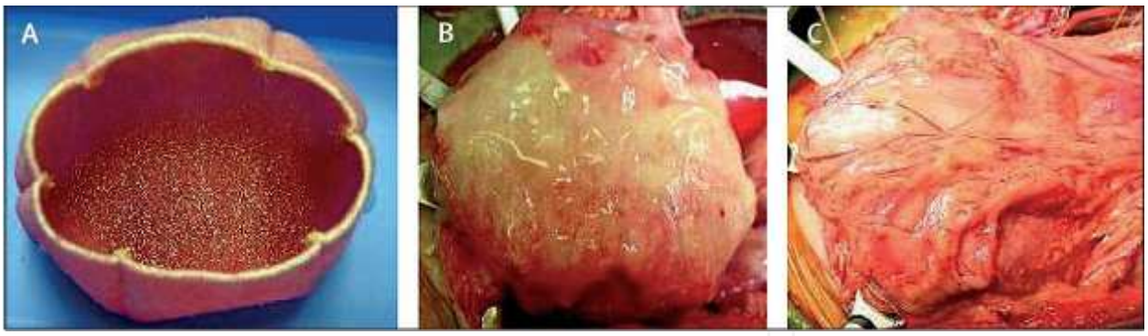

Fig. 18. Construction of engineered bladder scaffold seeded with cells (A) and engineered bladder anastamosed to native bladder with running 4–0 polyglycolic sutures (B). Implant covered with fibrin glue and omentum (C) (Ref 40)

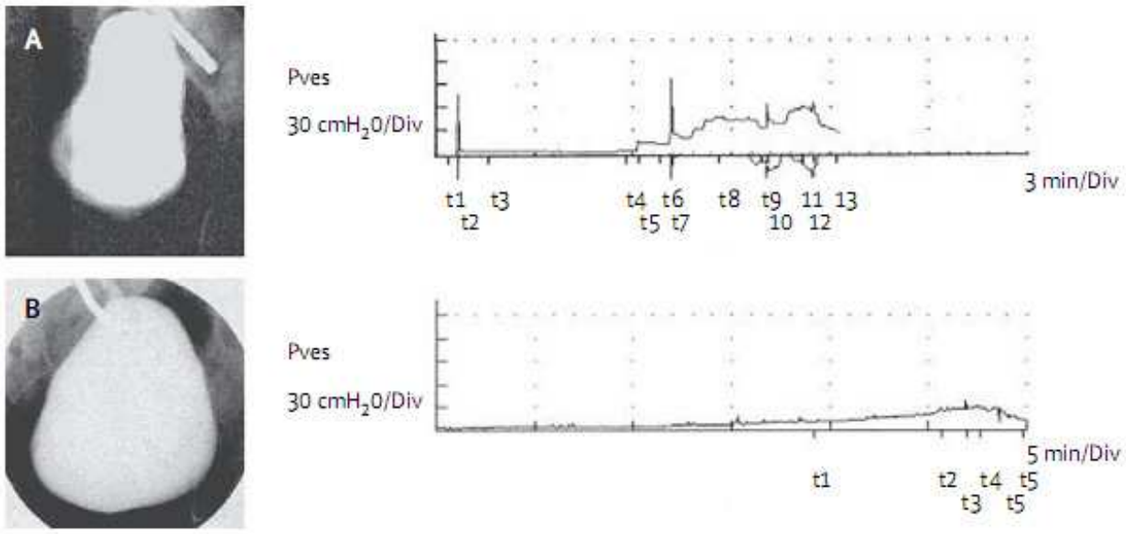

Fig. 19. Preoperative (A) and 10-months postoperative (B) cystograms and urodynamic fi ndings in patient with a collagen-PGA scaff old engineered bladder (Ref 40)

In another clinical study reported in the 2008 AUA, patients, who had received tissue engineered bladder showed increasing capacity and reduced intravesicular pressure. According to these reports, there is a clear reason to hope that the tissue engineered bladder can be utilized for a fully functioning neurogenic bladder. More indications about using this kind of biomaterial might be obtained in the near future.

3.2 Urethral reconstruction

The application of tissue engineering techniques for urethral reconstruction has been developed in recent years and the potential market for a tissue engineered solution for urethral stricture and abnormality will continue to increase in the near future.

3.2.1 Animal experiments

In animal experiments addressing urethral reconstruction, the first attempts used biomaterials alone. Among these experiments, most papers reported the application of SIS in animal urethroplasty. Many results were encouraging. Regenerated urethra contained a well-differentiated epithelium, underneath which was circular smooth muscle and abundant collagen and fibrous connective tissue. The only difference between the SIS-reconstructed urethra and normal urethra was the amount and size of the circular bundles of smooth muscle. However, several key points should be considered before using this kind of biomaterial. First, El-Assmy mentioned that locally prepared SIS and commercially available SIS may lead to the different results (41).This might be related to different pore sizes, which limit the infiltration and migration of cells. Second, the feasibility of using tabularized matrix for urethroplasty is still controversy although SIS has been proven to be useful for onlay urethroplasty. In Shokeir's study, a 3cm segment of the whole urethral circumference was excised and replaced by a tube matrix of the same length and width in 14 dogs. However, all dogs suffered a urethral fistula and/or stricture after the stent removal. This result demonstrated that a tube formed of matrix without seeded cells was not able to replace the long segment including the whole circumference of the canine urethra (42). Third, the length of urethral defect is another key point that should be considered during the urethroplasty. In order to investigate the maximum distance for normal tissue regeneration, Dorin et al. performed the tabularized urethroplasty in 12 male rabbits using acellular scaffold at varying lengths (0.5,1,2 and 3cm). The final result indicated that the maximal defect distance suitable for normal tissue formation using acellular grafts that rely on the native cells for tissue regeneration appears to be 0.5cm (43). Although other reports showed that the synthetic scaffold alone could be used for urethroplasty, the need ror a move to using cell-seeded scaffold is obvious.

In 2003, Bhargava has developed tissue-engineered buccal mucosa for use in substitution urethroplasty. Histologically, the matrix closely resembled the native oral mucosa after culturing for 2 weeks. A gradually increasing thickness of the epidermis and remodeling of the dermis could also be seen (44) (Fig 20). Subsequently, more cell-seeded scaffolds were used for urethral reconstruction in our center. Li et al. replaced urinary epithelial cells with oral keratinocytes seeded on BAMG to reconstruct a tissue-engineered urethra. Histological results showed that multiple layers of keratinocytes had formed at 2 and 6 months after the operation. Obvious margins between graft oral keratinocytes and host epithelium could be noticed in H&E sections (Fig 21b). Fu et al. used foreskin epidermal cell-seeded scaffolds to repair a urethra defect in a rabbit model. During following up, several layers of epidermal cells with abundant vessels in the submucosa were noticed. Moreover, immunofluorescence confirmed the survival of implanted epidermal cells at 1 month after procedure (45,46) (Fig 21a).

Recently, we have investigated the feasibility of constructing 3D structure urethra using multiple seeding cell types. It has also been hypothesized that building three-dimensional constructs in vitro prior to implantation would facilitate matrix vascularization in vivo and minimize the inflammatory response towards the matrix. Therefore, we seeded autologus corporal smooth muscle cells (CSMCs) and lingual keratinocytes into ACSM, using a static-dynamic seeding method. After being cultured 14 days, 6 scaffolds with two kind of cells

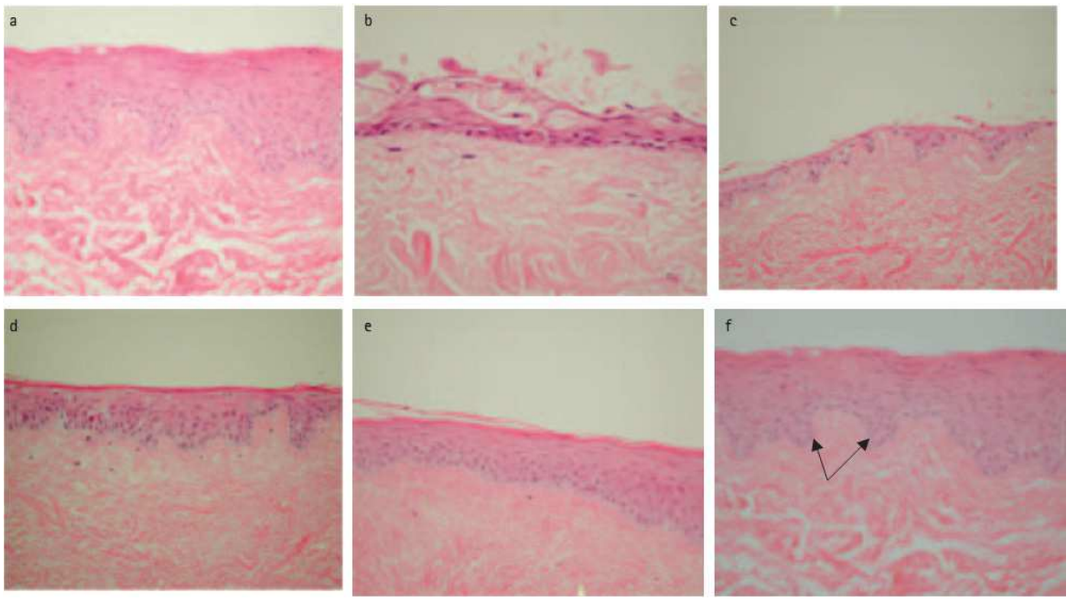

Fig. 20. buccal mucosa culture at a, the air–liquid interface; b, submerged; c, at day 1 ALI; d, at day 5 ALI; e, at day 8 ALI and with f, Protocol 2 (cells on same surface) (Ref 44)

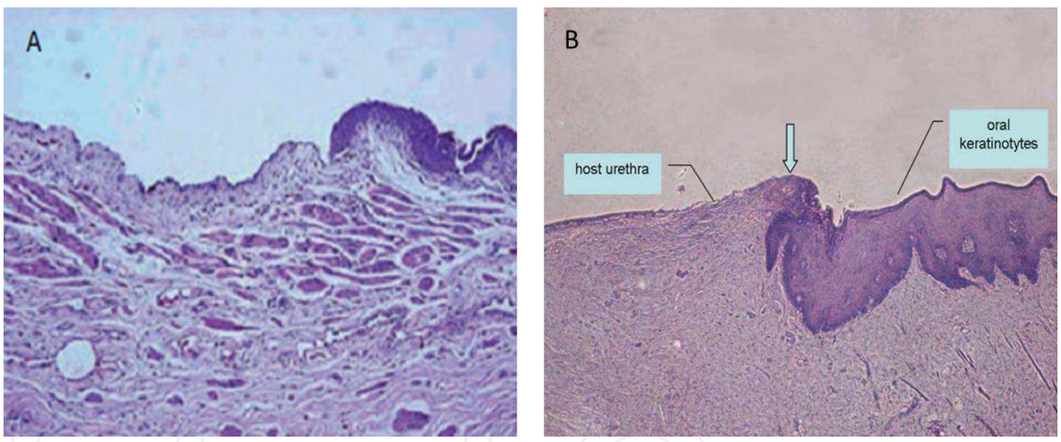

Fig. 21. Histological results of tissue engineering urethral after reconstruction. a. tissue engineering urethra using foreskin seeded scaffold; B, tissue engineering urethra using oral keratinocytes seeded scaffold. (Ref 44,45)

(Group C), 6 scaffolds with only lingual keratinocytes (Group B) and 6 matrices without cells (Group A) were used to repair a rabbit urethral defect. H&E staining of seeded ACSM showed several epithelial layers and well distributed CSMCs in the matrix. The maintenance of wide urethral caliber could be observed in Group C, while strictures were observed in groups A and B (Fig 22). Histologically, the retrieved urethra in group A showed fibrosis and inflammation during 6 months. A simple epithelial layer regenerated in group B but there was still no evidence of CSMCs growing into grafts during study period. A stratified epithelial layer and organized muscle fiber bundles were evident 6 months after implantation in group C (Fig 23). Our results demonstrated that lingual keratinocytes and CSMCs could be used as a source of seed cells for urethral tissue engineering. Using the

dynamic-static seeding method, a 3-D urethra could be constructed in vivo. It can provide us an alternative method to treat the urethral disease using tissue engineering techniquea.

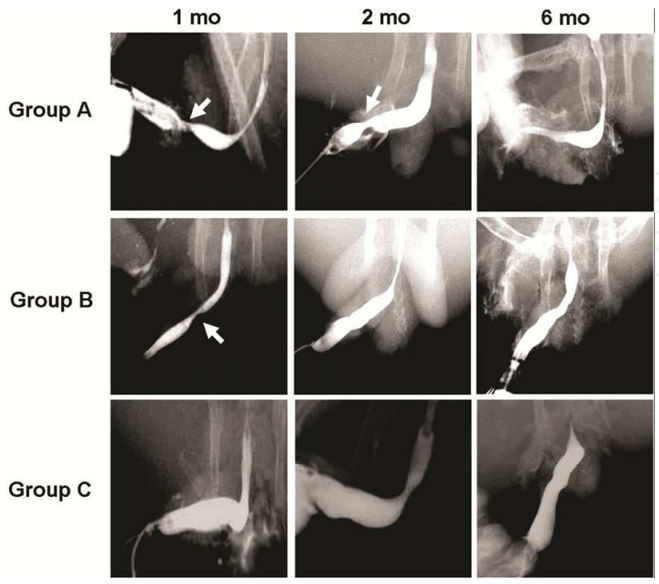

Fig. 22. Comparision of urethrography image in each group at 1,2,6 month after operation. The arrow indicates the stricture site of urethra

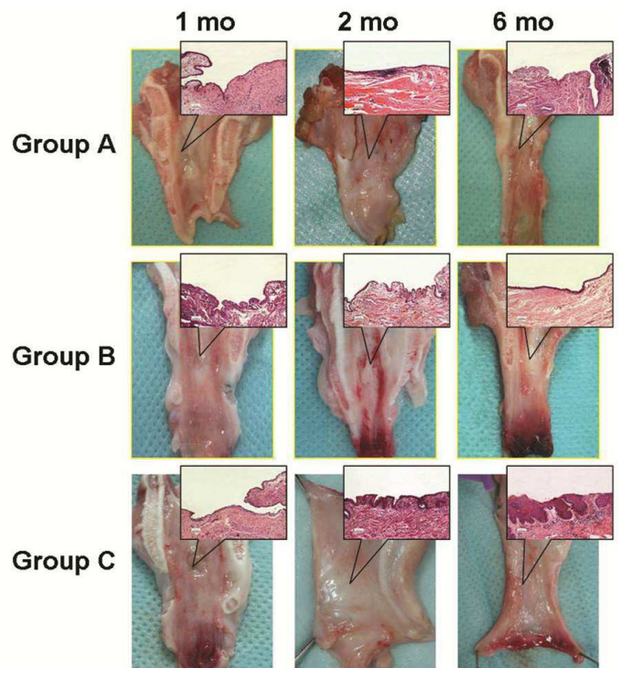

Fig. 23. Macroscopic inspection and H&E staining (inset) of retrieved urethrae in each group at 1,2,6 month after operation. In group A, a urethral stricture existed at every study time point. H&E staining did not show continuous epithelial layers but did show severe inflammation. In group B, strictures could be noticed by gross inspection. Only 1-2 epithelial layers were formed at 6 months after implantation. In group C, patent lumens without

strictures could be observed by the end of 6 months. Meanwhile, multilayer squamous epithelial layers covered the surface of the urethra.

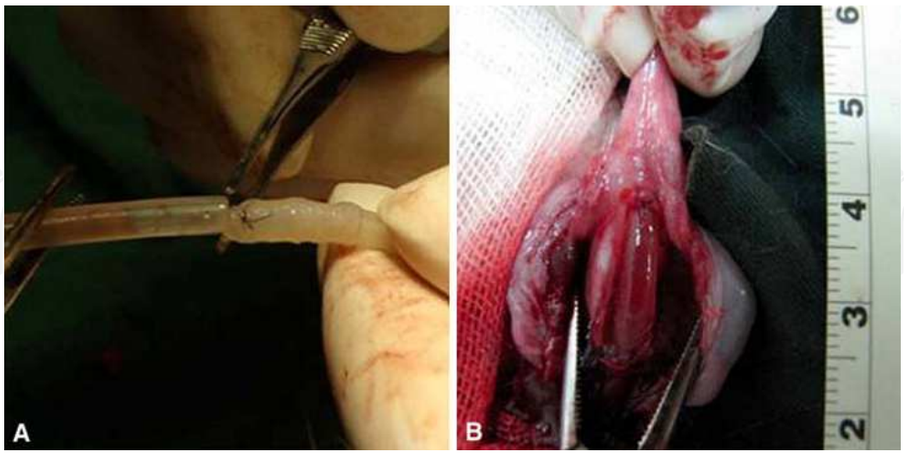

Fig. 24. a. Tubular tissue was gently everted so that the mesothelium lined the lumen. b The everted tubular tissue was interposed and anastomosed as urethral graft (Ref 48)

According to this result, the authors concluded that autologous tissue grown within the recipients' peritoneal cavity can be used successfully for tabularized urethral reconstruction (48). In addition, synthetic matrix combined with seeded cells has also been used for urethroplasty. In Selim's study, the optimal sterilization and cell seeding method for synthetic biomaterials in urethral reconstruction has been investigated. In their study, both PAA and γ-irradiation appear to be suitable methods for sterilizing PLGA scaffolds. And the sterilized PLGA 85:15 is a promising material for tissue engineering urethral reconstruction (49).

3.2.2 Clinical application

Up till now, many urologists have reported successful outcomes of urethral reconstruction using tissue engineering techniques. Most reports have been focused on treating urethral strictures using SIS. Among them, most results were satisfactory (Table 2) (50-56). In the report of Fiala et al., fifty patients with urethral strictures received urethroplasty using SIS. During post-operative follow-up, clinical, radiological, and cosmetic findings were excellent in 80% patients. No complications, such as fistulae, wound infections, or rejection were observed. This is so far the largest reports about using SIS for urethral reconstruction, in terms of numbers of patients. Their results were more satisfacory even than traditional urethral reconstruction using buccal or lingual mucosa for such low complication rate. In our center, we have also used SIS patch to undergo in 16 male patients with urethral strictures. The average length of strictures was 4.6 cm, ranging from 3.5-6 cm (Fig 25). After the operation, urethrography showed a wide patent urethra in all patients. The mean Qmax increased obviously from 3.8ml/s to 25ml/s. Only one patient needed urethral dilation due to the decreasing of Qmax at the end of 5 months. During follow-up, routine urethroscopy was performed in all patients. At the end of 4 weeks after operation, SIS could be easily noticed in the urethral lumen. However, the implanted graft could not be identified from the normal urethra 38 weeks after operation (Fig 26). The HE staining of biopsy showed that

stratified squamous epithelial layers had grown on the SIS implanted site, which was similar to normal urethral mucosa (Fig 27). According to these clinical experiences, the use of an acellular matrix SIS for urethroplasty should only be done when the length of urethral stricture is short. Patients with a bulbar urethral stricture are more suitable than those with a urethral stricture in other sites. Of course, the condition of urethral plate should also be considered before using SIS. We believe that urethroplasty using tissue engineered scaffold can achieve a satisfactory outcome that is similar to the gold-standard procedure *provided optimal patients are selected*.

Author	Date	Patient number	SIS type	SIS layer	Stricture length	Recurrence
Mantovani F, et al	2003	1	patch	1	>10cm	none
Le Roux JP	2005	9	tube	1	2-4cm	66.7%(6/9)
Hauser D, et al.	2005	5	patch	4	3.5-10cm	80%(4/5)
Sievert KD, et al.	2005	13	patch	4	4-10cm	30.7%(4/13)
Donkov II,et al	2006	9	patch	4	4-6cm	11.1%(1/9)
Palminteri E,et al	2007	20	patch	4	3-7.7cm	15%(3/20)
Fiala R,et al	2007	50	patch	4	4-14cm	20%(10/50)
Farahat,YA	2009	10	patch	1	0.5-2cm	20%(2/10)

Table 2. Recoder of using SIS for urethral reconstruction in clinic

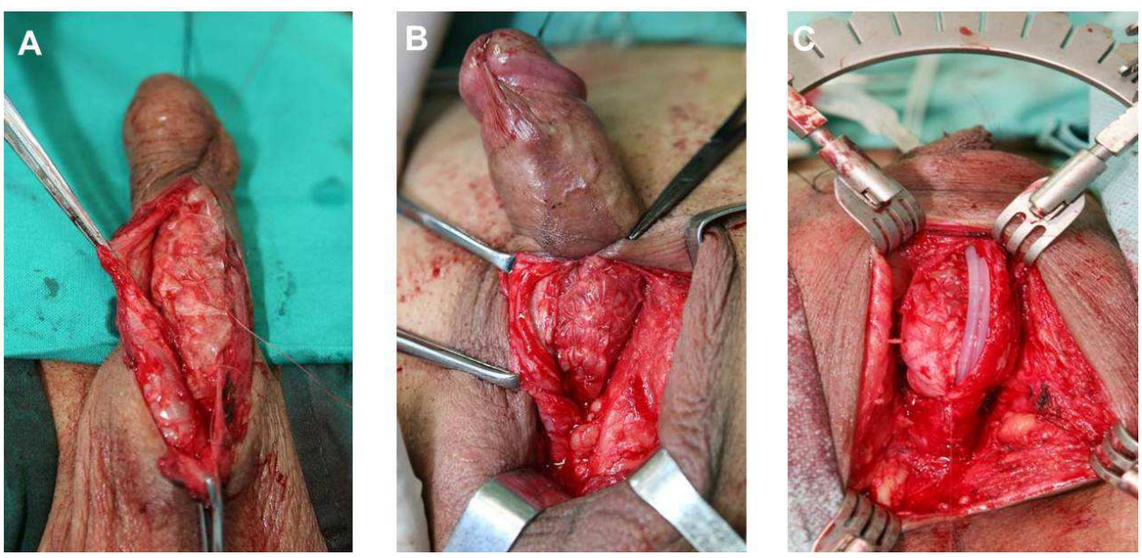

Fig. 25. Application of SIS in urethroplasty.
a. penile urethral stricture; b.bulbopenile urethral stricture; c.bulbar urethral stricture.

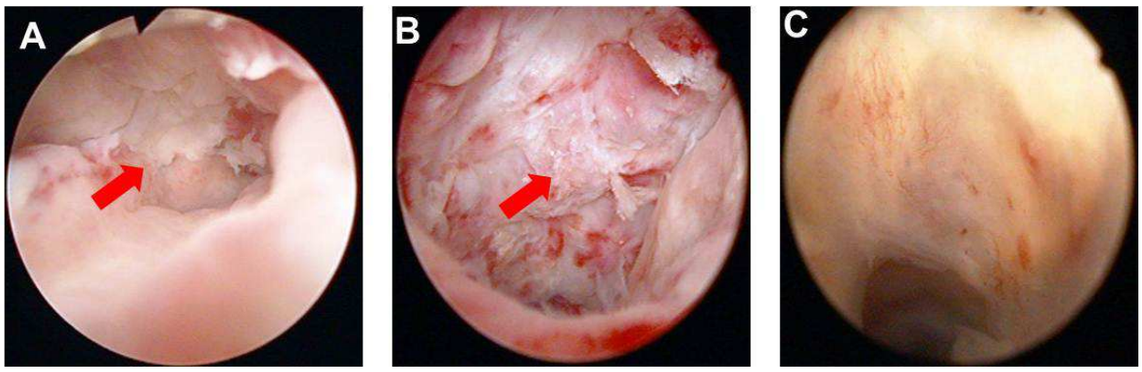

Fig. 26. Urethroscopy after the urethral reconstruction using SIS graft.Arrow headed the implanted site. a:4 weeks after op; b: 6 weeks after op; c: 38 weeks after operation.

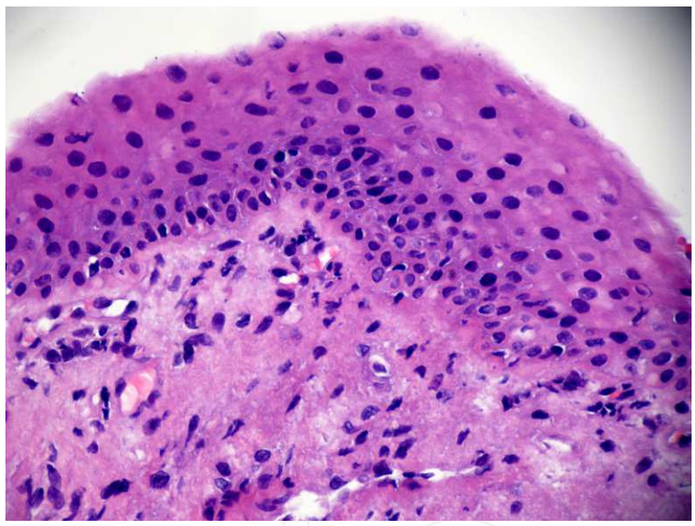

Fig. 27. The HE staining of biopsy showed that stratified squamous epithelial layers have grown on the SIS implanted site.

Cell-seeded scaffolds have also been used for urethral reconstruction in some patients. Based on the previous reports mentioned above, Bhargava et al. useed autologous tissue-engineered buccal mucosa to treat five patients with urethral strictures secondary to lichen sclerosis. After the intimal operation, one patient had complete excision of the grafted urethra and one required partial graft excision. The other three patients required some form of instrumentation although endoscopic appearance showed a patent urethra with the implanted graft in situ (57). Recently, Atala et al. reported that using cell-seeded synthetic tubularized scaffolds to repair urethral defects in five boys. At the end of follow-up, some satisfactory results were obtained. The median end Qmax was 27.1 ml/s, and serial urethrographic and endoscopic studies showed the maintenance of wide calibres without strictures (Fig 28) (58).

Fig. 28. A cell-seeded graft sutured to the normal urethral margins(Ref 59)

4. Challenges and risks

Based on previous studies, the potential market for a tissue-engineered solution for dysfunctional bladders and small contracted or inflamed bladders is probably far too small for commercial exploitation. The only two potential indications for commercial-scale tissue engineering lower urinary tract tissue are bladder carcinoma and urethral stricture. Cell-seeded scaffolds will probably be further investigated and applied in clinics. hree-dimensional structures and the use of bioreactors will also be more and more popular in tissue engineering research for lower urinary tract reconstruction.

However, several problems need to be solved. For example, the ethical problems about the implanted matrix (and where it is obtained) needs to be further discussed. The potential for carcinogenic problems arising form the use of stem cells is not clear. Optimal methods of cell labeling (for research) still needs to be improved.

Nevertheless, there is no doubt that tissue engineering techniques for lower urinary tract reconstruction will themselves become the gold-standard in the near future. A substantial commercial market will continue to grow and more patients will obtain benefit from this technique.

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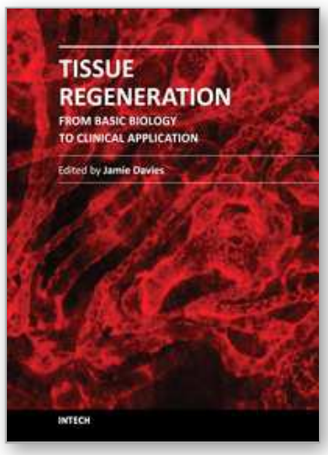

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When most types of human tissue are damaged, they repair themselves by forming a scar - a mechanically strong 'patch' that restores structural integrity to the tissue without restoring physiological function. Much better, for a patient, would be like-for-like replacement of damaged tissue with something functionally equivalent: there is currently an intense international research effort focused on this goal. This timely book addresses key topics in tissue regeneration in a sequence of linked chapters, each written by world experts; understanding normal healing; sources of, and methods of using, stem cells; construction and use of scaffolds; and modelling and assessment of regeneration. The book is intended for an audience consisting of advanced students, and research and medical professionals.

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