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Review

Tissue engineering for urinary tract reconstruction and repair: Progress and prospect in China

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Abstract Several urinary tract pathologic conditions, such as strictures, cancer, and obliterations, require reconstructive plastic surgery. Reconstruction of the urinary tract is an intractable task for urologists due to insufficient autologous tissue. Limitations of autologous tissue application prompted urologists to investigate ideal substitutes. Tissue engineering is a new direction in these cases. Advances in tissue engineering over the last 2 decades may offer alternative approaches for the urinary tract reconstruction. The main components of tissue engineering include biomaterials and cells. Biomaterials can be used with or without cultured cells. This paper focuses on cell sources, biomaterials, and existing methods of tissue engineering for urinary tract reconstruction in China. The paper also details challenges and perspectives involved in urinary tract reconstruction.

1. Introduction

Repair and reconstruction of urinary tracts are major issues in urology. A variety of techniques, such as resection of lesion, reconstruction with autologous tissue, and replacement with allografts, are used to manage these cases [1,2]. Resection of lesions is not a practical option when extensive lesions exist, and it has lower success rates when compared with other procedures. However, the application of autologous transplantation is limited by donor site availability. Simultaneously, longer grafts induce higher donor site morbidity such as pain, swelling, post-operative infection, hemorrhaging, and even deformity [3,4]. Additionally, donor organ shortage and tissue rejection block allogeneic transplantation in practical applications [2]. Therefore, researchers increasingly focused on tissue engineering to repair urinary tract defects. Advances in tissue engineering

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can overcome the drawbacks of traditional therapeutic strategies and provide an alternative approach for urinary tract reconstruction and repair. Tissue engineering is a component of regenerative medicine that can remedy severe defects and restore normal functioning of tissues [5]. In the last decade, numerous strategies for urinary tract regeneration were proposed in the field of tissue engineering. This study involved a review of cell sources, materials in urinary tract tissue engineering, and implantation techniques in China. The study also discusses the challenges and prospects of urinary tract tissue engineering.

2. Cell sources

A controversy is associated with the necessity of cell seeding in tissue-engineered urinary tracts. Most studies demonstrated that cell-free scaffolds could result in fibrosis post-operatively [6,7]. With respect to extensive lesions, cell seeding is proposed to prevent scar formation [8] and improve tissue regeneration [9,10]. A potential mechanism involves the promotion of rapid formation of urothelial barriers by cell-seeded scaffolds, and this can prevent urinary irritation. Urine is toxic to progenitor or stem cells recruited from normal adjacent tissues. Additionally, urine is also a major factor of inflammatory infiltration that results in fibrous tissue deposition. Cell sources used in urinary tract reconstruction include differentiated primary cells and stem cells (Fig. 1).

Autologous urothelial cells (UCs) are obtained from a urinary bladder that is commonly used in urinary tract reconstruction. A bladder biopsy is a commonly used method for harvesting bladder urothelial cells. A study by Romagnoli et al. [11] repaired posterior hypospadias with urethral epithelium harvested from a biopsy of urethral mucosa. However, these protocols are invasive due to surgical intervention and trauma to the bladder and urethra. A few researchers obtained urothelial cells from bladder washes and urine. The cells isolated from urine present the characteristics of normal bladder cells and are potentially promising for urethral reconstruction [12—14]. Epidermal cells can be readily obtained from foreskin with only minor donor morbidity. These cells can be easily incubated and are sufficient in quantity when they are seeded on acellular matrix [15]. Epithelial cells obtained from autologous oral are also adaptable for urinary tract substitution since their structure is similar to that of urothelium [16]. Buccal mucosa possesses non-keratinized a stratified squamous epithelium, which is highly resilient when exposed to greater mechanical abrasion. Oral keratinocytes are obtained by mechanically isolating the epidermis, and a traditional feeder dependent approach can facilitate expansion of oral keratinocytes. A biopsy can also induce donor morbidity. However, this approach is less invasive when compared with other epithelial cell sources from the urethra and bladder [17]. Smooth muscle cells (SMCs) can be isolated from the bladder, and these cells increase the elasticity of caliber and prevent wall adhesions and collapsing via rapid formation muscle layer [18]. Furthermore, transforming growth factor-β1 (TGF-β1) secreted by SMCs can promote angiogenesis [19]. Fibroblasts obtained from dermal tissue can secrete a series of cytokines and collagen that increase mechanical properties of the grafts and promote keratinocyte expansion [20]. In renal tissue engineering, the presence of proximal tubule cells (PTCs) is crucial in maintaining kidney functioning and PTCs can be

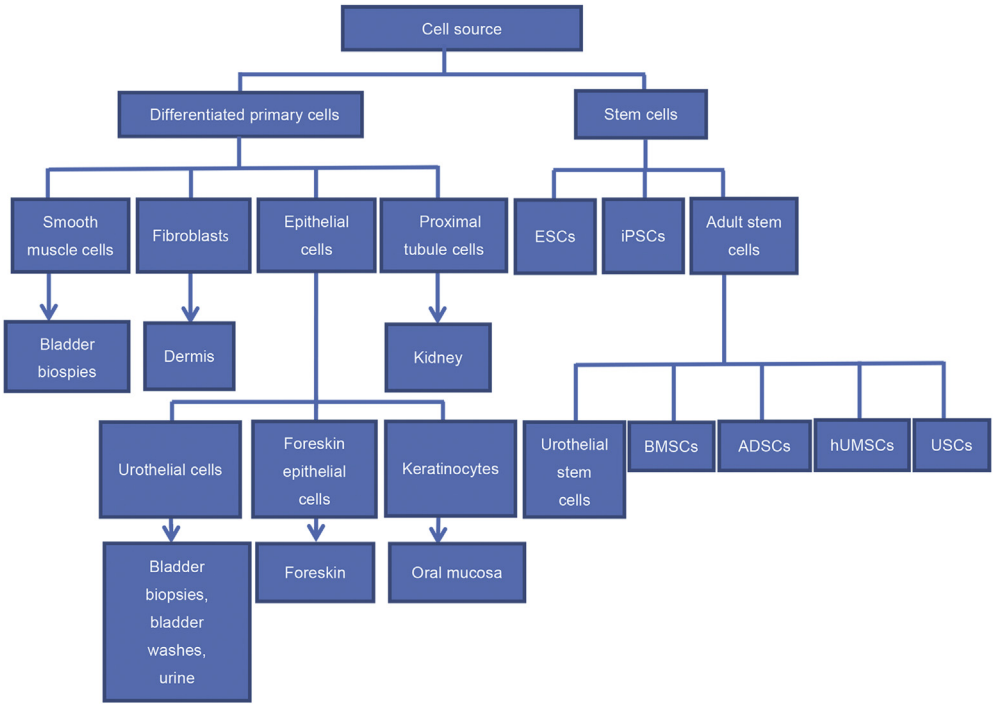

Figure 1 Cell sources in urinary tract reconstruction. ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells; BMSCs, bone marrow stem cells; ADSCs, adipose tissue-derived stem cells; hUMSCs, human umbilical cord-derived mesenchymal stem cells; USCs, urine-derived stem cells.

harvested from normal or diseased kidney tissues [21]. Primary PTC cultures are more representative than immortalized cell lines. However, primary kidney cells lose certain expressions of important genes during passaging and possess limited proliferation potential [22].

Stem cells are considered as undifferentiated cells and can differentiate into a wide variety of differentiated cells [23]. Embryonic stem cells (ESCs) are theoretically considered as an ideal source of seeded cells due to multiple differentiation potential. However, the application is restricted by a major concern, namely the origin of their embryos and the probability of tumor formation [24]. Additionally, ESCs can differentiate into urothelial cells when treated with all-trans retinoic acid *in vitro* [25]. The ability to induce ESCs into urothelium *in vitro* holds significant implications for urinary tract reconstruction [26]. A controversy with respect to the application of ESCs exists. However, differentiation control is required prior to their use in regenerative medicine. Induced pluripotent stem cells (iPSCs) are an alternative to ESCs and possess important features similar to ESCs, such as proliferation and cloning capacities. Specifically, iPSCs can be generated from autologous adult somatic cells through a viral vectors approach to express the key factors SOX2, c-Myc, OCT4, and Klf4 [27]. Osborn et al. [26] developed an efficient *in vitro* induction protocol for the induction of iPSCs into the urothelium to provide a novel source of bioengineered urothelium.

Adult stem cells (ASCs) from the tissues and organs of an adult, such as bone marrow, skin, and fat, constitute resident stem cells. These cells maintain homeostasis and regeneration of tissues after a minor injury due to proliferation and differentiation abilities [28]. Additionally, ASCs are autologous and do not cause evident immune rejection. Moreover, these cells bypass ethical obstacles and are relatively easy to isolate. Urothelial stem cells, isolated from the ureter, urethra, and bladder possess a self-renewal feature and can differentiate into urothelium. A few reports demonstrated that urothelial stem cells in mice express sonic-hedgehog and p63 proteins [29,30]. A study by Larsson et al. [31] isolated human urothelial cells and demonstrated that these cells possess the characteristics of clonogenicity, self-renewal, and morphological differentiation. Bone marrow stem cells are obtained from bone marrow to generate SMCs and UCs [32,33]. Nevertheless, a major concern relates to a small amount of stem cells in bone marrow that entails significant time to expand *in vitro* to obtain adequate cells. Additionally, the process of isolating can lead to discomfort because of bone marrow aspiration. Adipose tissue-derived stem cells (ADSCs) are similar to mesenchymal stem cells from bone marrow stroma with a stable undifferentiated and high proliferative status *in vitro*. They can differentiate into multiple cell lineage including SMC and UC [34]. The coculturing of ADSCs with UCs or treated with all-trans retinoic acid leads to the differentiation of ADSCs into UCs [35,36]. Specifically, in a previous study, ADSCs maintained their phenotype for 2 weeks *in vitro* prior to implantation in nude mice [37]. These findings suggested that ADSCs can be an ideal source for urinary tract reconstruction due to a minimally invasive procedure and the presence of abundant quantities in adipose tissue [38]. Human umbilical cord-derived mesenchymal stem cells (hUMSCs) are obtained from fresh cord blood and may be an excellent source of ASCs due to their multipotent differentiation ability [39,40]. Importantly, hUMSCs transplants exhibit less immunologic rejection when compared to other ASCs, and this property makes them ideal candidates for urinary tract repair and reconstruction [41]. Urine-derived stem cells (USCs) can be isolated from urine and do not involve a risk of teratoma formation. Furthermore, USCs possess significant proliferative capacity and can produce a considerable number of cells from a small amount of cells [42,43]. The treatment of USCs with appropriate media leads to their differentiation into multiple cell types including UCs and SMCs that constitute major seeded cells in urinary tract reconstruction [44].

3. Biomaterials

Biomaterials control the biological microenvironment and provide a three-dimensional (3D) space for regenerated tissues [45]. The functions of scaffolds used in tissue engineering are dependent on the properties of selected biomaterials [46]. Thus, in addition to the role of delivery vehicles to transport nutrients and wastes [47], the properties of the selected biomaterials must also include the functions of promoting proliferation, differentiation, and attachment [48]. Ideal biomaterials should possess a controlled degradation rate without toxicity. A variety of biomaterial scaffolds were used for urinary tract reconstruction. These are classified into two major types, namely synthetic and natural biomaterials (Fig. 2). The first type includes non-degradable materials (such as silicone and polyurethane) and biodegradable polymers such as polylactic acid (PLA), polyglycolic acid, and polylacticcoglycolic acid (PLGA). Non-degradable materials used in urinary tract reconstruction exhibited poor results with the occurrence of a variety of complications such as calcification and fistulae [49]. In contrast, biodegradable scaffolds are mostly used in urinary tract reconstruction as they exhibit better results [50]. Natural materials include the following two groups: natural polymers (mostly collagen) and acellular matrices

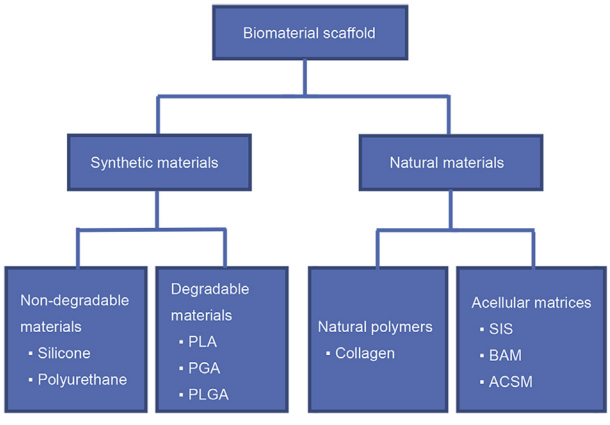

Figure 2 Biomaterials in urinary tract reconstruction. PLA, polylactic acid; PGA, polyglycolic acid; PLGA, polylacticcoglycolic acid; SIS, small intestinal submucosa; BAM, bladder acellular matrices; ACSM, acellular corpus spongiosum matrices.

obtained from cadaveric or animal organs via enzymatic, physical, or chemical methods [51]. A study by Nuininga et al. [52] repaired urethral lesions with cross-linked collagen and compressed collagen structures in rabbit models, and retrograde urethrography showed a normal caliber at 2 weeks postsurgery. The most commonly used acellular tissue matrices in urethral reconstruction are small intestinal submucosa (SIS), bladder acellular matrices (BAM), and acellular corpus spongiosum matrices. It is necessary to choose between synthetic and natural scaffolds prior to the use of a scaffold in urinary tract reconstruction. Synthetic scaffolds are advantageous as they are easily available, highly reproducible, and possess excellent mechanical properties. However, synthetic scaffolds involve the absence of cytokines and extracellular matrix (ECM) proteins that are major factors in regulating cellular proliferation and differentiation and mimic the microenvironment [53]. A few synthetic polymers can result in inflammatory responses *in vivo* and generate acidic products that impair cellular growth [54]. Natural scaffolds reserve 3D structure and bioactive factors of the original tissue and represent biocompatible and biodegradable materials [55]. However, natural scaffolds are characterized by the absence of structural strength and biochemical properties that are difficult to control. These properties are associated with fibrosis and contracture when implanted into urethra [56].

4. Tissue engineering approaches for renal repair and reconstruction

The kidney is an important organ with multiple functions such as filtration function, erythropoietin secretion, and regulation of electrolytes and pH. The filtration function is the most important function among these functions. The filtration function is mainly dependent on a nephron that is composed of various types of cells and complicated vascular structures. Additionally, it is also necessary to consider other functions when researchers construct fully functional kidneys. Tissue engineering approaches for renal repair and reconstruction as reported in China are listed in Table 1.

Renal function can be ameliorated via cell-only approaches in renal regeneration. Pluripotent stem cells (PSCs) can differentiate into different cell types and form heterogeneous tissues due to the self-assembling approach [57,58]. Specifically, PSCs are composed of ESCs and iPSCs that may provide alternative cell types for kidney regeneration. International studies reported that PSCs can generate intermediate mesoderm and metanephric mesenchyme cells that constitute nephron progenitor cells [59]. Despite these advances, this approach has not succeeded in constructing multi-function kidneys due to the absence of other functions in the regenerated kidneys.

The mesenchymal stem cells (MSCs) play a vital role in preserving renal parenchymal integrity from acute damage. Possible mechanisms of MSCs include the promotion of tubular epithelium regeneration, secretory functions, and peri-tubular capillary regeneration. Chen et al. [60] used ADSC to repair renal function following acute ischemia—reperfusion injury. The results indicated that ADSC could minimize kidney damage through the suppression of inflammatory response and promotion of angiogenesis. Furthermore, MSCs derived from human umbilical cord (UC-MSCs) possess stem cell properties and represent a more abundant and safer source for stem cell therapy. Cao et al. [61] demonstrated that UC-MSCs can ameliorate renal functions in ischemia/reperfusion-induced acute renal failure via replacement of injured tissues and secretion relative cytokines. Growth factors, such as vascular endothelial growth factor (VEGF), epidermal growth factor, and hepatocyte growth factor (HGF), can increase the therapeutic effect in the therapy of acute kidney injury (AKI) [62]. Yuan et al. [63] utilized human embryonic MSC combined with VEGF to heal cisplatin-injured renal failure. Chen et al. [64] reported a similar study to evaluate the effect of HGF modified human umbilical cord-derived mesenchymal stem cells on AKI, and the results further confirmed the vital role of growth factors on stem cell therapy. Exosomes are microvesicles released from various cells and are present in extracellular space. Exosomes include a number of components such as mRNA, microRNA and proteins. Previous studies demonstrated that hUMSC-excreted exosomes reduce cisplatin-induced oxidative stress by decreasing the formation of products and promoting cell proliferation [65].

The kidney is an organ with a complex structure and functions, and it is composed of multiple cell types and a

Table 1 Tissue engineering approaches used for renal reconstruction in China.

Research	Animals for modeling	Cell types	Biomaterial/ artificial tissue	Kidney damage	Duration of observation
Chen et al., 2011 [60]	Rats	ADSCs	—	Acute renal failure	72 h
Cao et al., 2010 [61]	Rats	hUMSCs	—	Acute renal failure	72 h
Yuan et al., 2011 [63]	Mice	VEGF-modified ESCs	—	Acute renal failure	72 h
Chen et al., 2011 [64]	Rats	FGF-modified hUMSCs	—	Acute renal failure	72 h
Zhou et al., 2013 [65]	Rats	Exosomes released by hUMSCs	—	Acute renal failure	120 h
Yu et al., 2014 [67]	Rats	—	KAM	Partially nephrectomized kidneys	6 weeks
Guan et al., 2015 [66]	Rats	ESCs	KAM	Nephrectomized kidneys	2 weeks

ADSCs, adipose tissue-derived stem cells; ESCs, embryonic stem cells; FGF, fibroblast growth factor; hUMSCs, human umbilical cord-derived mesenchymal stem cells; KAM, kidney acellular matrices; VEGF, vascular endothelial growth factor.

complicated vascular network. Guan et al. [66] introduced a decellularization technology to produce kidney acellular matrices from porcine that retained the 3D architecture and the vascular tree. Further studies were not performed to recellularize the scaffold with autologous cells. However, this study could have important implications for kidney regeneration since the size and architecture of the kidneys in the studies was similar to those of human kidneys. Yu et al. [67] produced a decellularized kidney scaffold from rats and implanted the same into a kidney after partial nephrectomy. Renal functions were restored at six weeks post-implantation because of physical support as well as biochemical cues of the kidney scaffold. However, the cell-free scaffolds are not feasible in creating transplantable kidneys due to the absence of residual cells. Studies investigate the use of scaffolds combined with stem cells to generate a whole kidney. Guan et al. [68] utilized decellularized kidney scaffolds from rats and mice ESCs to recellularize kidney scaffolds. The fore-mentioned scaffolds were orthotopically implanted in rats, and the structure and major functions exhibited by the recellularized kidney were similar to those of native kidneys. This approach indicated that it is possible to reconstruct transplantable kidneys free from immune response.

5. Tissue engineering approaches for ureteral reconstruction

Research focused on ureteral tissue engineering is limited when compared with that focused on other organs in the urinary system. Table 2 shows tissue engineering approaches for ureteral reconstruction as reported in China. Most international studies used bare scaffolds in ureteral tissue engineering and demonstrated poor results due to fibrosis and inflammation, which indicated the necessity of cell-seeding [69,70]. Two crucial cell types in the ureter include the following: human UCs that form a permeability barrier to prevent the reabsorption of toxic urine, and SMCs that are responsible for contractile function and compliance of the ureter through smooth muscle layers. Several researchers chose autogenous UCs as seeded cells to prevent chronic immune response and fibrosis [71–73]. The main difference in the three fore-mentioned studies involves the method used to obtain ideal biomaterials with mechanical properties, biocompatibility, and low toxicity. Fu et al. [71] and Xu et al. [72] utilized poly-L-lactic acid (PLLA) combined with collagen and decellularized matrices, respectively, to achieve composited scaffolds. These composited scaffolds provide a favorable surface for cell proliferation and attachment and possess good mechanical properties. Another study transplanted PLLA stents into subcutaneous tissues of rats to form connective tissue capsules on surfaces and these connective tissue capsules were decellularized and further recellularized [73]. However, the disadvantage of autogenous cells in ureteral tissue engineering is that biopsies do not harvest sufficient cells especially in patients with urologic cancer or extensive tissue damage. Under these types of conditions, stem cells can constitute the ideal origin for ureteral tissue engineering. Liao et al. [9] seeded MSCs and SMCs onto bladder acellular matrices (BAM), and the cell-seeded grafts were transplanted into the omentum of a rabbit prior to ureteral reconstruction. Furthermore, ADSCs possess the potential to differentiate into epithelial cells and SMCs. Moreover, ADSCs are already used to differentiate SMCs in tissue engineering urethra [10]. Additionally, Shi et al. [37] differentiated ADSCs into urothelial lineage and then seeded the same on PLA/collagen scaffolds. A similar study constructed a tissue-engineered ureteral by seeding ADSCs and SMCs on bladder submucosa matrix (BSM). Multilayered urothelium and neovascularization are observed in the graft [74].

6. Tissue engineering approaches for bladder reconstruction

A variety of studies on bladder reconstruction were reported in China (Table 3). The cell-free scaffolds approach can be an ideal strategy for bladder reconstruction because this strategy is simple and does not require cell harvesting and culture *in vitro*. With respect to the scaffolds, BAM and SIS are widely used in bladder reconstruction. Zhu et al. [75] and Wang and Liao [76] evaluated the potential use of BMA and SIS, respectively, as bioscaffolds in tissue engineering bladder in the rabbit model. The results of both studies showed that regenerated bladders possessed similar histologic and functional properties. Zhao et al. [77] produced a bilayer scaffold using a silk fibroin combined

Table 2 Tissue engineering approaches for ureteral reconstruction in China.

Research	Animals for modeling	Cell types	Biomaterial/ artificial tissue	Repaired length	Duration of observation
Fu et al., 2012 [71]	Mice	Ureteral epithelial cells	PLLA-collagen nanofibrous	—	2 weeks
Xu et al., 2012 [73]	Rats	Bladder epithelial cells	PLLA	0.9 cm	3 weeks
Liao et al., 2013 [9]	Rabbits	BMSCs + SMCs	BAM	4 cm	16 weeks
Zhao et al., 2012 [10]	Rabbits	Mesothelial cells	BAM	3 cm	16 weeks
Shi et al., 2012 [37]	Mice	ADSCs induced epithelial cells	PLA/collagen scaffolds	—	2 weeks
Meng et al., 2015 [74]	Rabbits	ADSCs + SMCs	BAM	4 cm	16 weeks

ADSCs, adipose tissue-derived stem cells; BAM, bladder acellular matrices; BMSCs, bone marrow stem cells; PLLA, poly-L-lactic acid; SMCs, smooth muscle cells.

Table 3 Tissue engineering approaches for bladder reconstruction in China.

Research	Animals for modeling	Cell types	Biomaterial/artificial tissue	Repaired surface defect	Duration of observation
Zhu et al., 2011 [75]	Rabbits	—	BAM	—	24 weeks
Wang and Liao, 2014 [76]	Rabbits	—	SIS	10 cm × 3 cm × 3 mm	24 weeks
Zhao et al., 2015 [77]	Rats	—	BAM-silk fibroin (SF)	10 mm × 10 mm	12 weeks
Jiang et al., 2015 [78]	Rabbits	—	VEGF-loaded nanoparticles-modified BAM	2 cm × 3 cm	12 weeks
Xiong et al., 2015 [79]	Swine	—	VEGF-loaded nanoparticles-modified BAM	35%—50% of the bladder	12 weeks
Chen et al., 2014 [80]	Rats	—	FGF modified BAM	A diameter of 1 cm	12 weeks
Chen et al., 2010 [81]	Rats	—	FGF modified collagen scaffolds	Half of bladder upper	12 weeks
Zhou et al., 2013 [82]	Rats	—	PDGF and VEGF modified BAM	4 cm × 5 cm	24 weeks
Jiang et al., 2016 [83]	Rabbits	—	FGF and VEGF modified BAM	2 cm × 3 cm	12 weeks
Chen et al., 2011 [85]	Swine	VEGF modified EPCs	BAM	40% of the bladder	24 weeks
Zhang et al., 2004 [86]	Mice	SMCs + UCs	SIS	1 cm × 1 cm	12 weeks
Zhu et al., 2010 [87]	Rabbits	ADSCs	BAM	1.5 cm × 1.5 cm	24 weeks
Zhe et al., 2016 [88]	Rats	ADSCs	BAM	—	14 weeks
Yuan et al., 2013 [89]	Canine	hUMSCs	BAM	40% of the bladder	12 weeks

ADSCs, adipose tissue-derived stem cells; BAM, bladder acellular matrices; EPCs, endothelial progenitor cells; FGF, fibroblast growth factor; hUMSCs, human umbilical cord-derived mesenchymal stem cells; PDGF, platelet-derived growth factor; SIS, small intestinal submucosa; SMCs, smooth muscle cells; UCs, urothelial cells; VEGF, vascular endothelial growth factor.

bladder acellular matrix and proved that these biomaterials promote vessel and nerve regeneration. However, the cell-free scaffolds approach is unsuitable for larger bladder regeneration. An increase in bladder defects leads to a significant decrease in the ability of a vascular network to supply sufficient nutrients for the cells, and the remaining bladder provides insufficient structural support. In order to overcome these limitations, increasing attention is focused on a method of promoting smooth muscle regeneration and neovascularization. As is widely known, growth factors play vital roles in the bladder development and regeneration. Although a natural ECM retains bioactive factors, the amount of factors is insufficient for bladder regeneration. Specifically, VEGF is a crucial bioactive factor that regulates an adjacent endothelial cell to migrate and proliferate *in situ* and promote vasculature formation. Bioscaffolds combined with VEGF to promote neovascularization are widely used in bladder regeneration [78,79]. Furthermore, FGF and platelet-derived growth factor-BB (PDGF-BB) are used to promote smooth muscle regeneration and accelerate neovascularization of a bladder [80—82]. A few researchers incorporated bioscaffolds with multiple growth factors, and the results revealed that multiple growth factors exhibited a significantly favorable performance in neovascularization and inhibition of contracture when compared with a single growth factor [82,83].

Cell-seeded technology on biomaterials and synthetic polymers are introduced to avoid immunological rejection and improve the regeneration process. Autologous endothelial cells can act as an alternative to rapidly establish blood supply in addition to the strategy of incorporating growth factors into biomaterials. However, endothelial progenitor cells are more desirable than endothelial cells because of their less invasive harvesting procedure [84,85]. Additionally, UC and SMC are crucial cells in the generation of an ideal bladder due to their roles in urinary barrier and mechanical support. Internationally, studies demonstrated that biomaterials seeded with both cells can generate an organized bladder albeit not in cell-free biomaterials [86]. This approach is not suitable for patients with bladder cancer despite advances in seeding materials with UC and SMC. Therefore, increasing attention is focused on stem cells as alternative cell sources for bladder reconstruction. ASCs harvested from bone marrow or adipose or umbilical cord tissues can overcome these limitations. However, the use of MSCs is not always acceptable because of an invasive harvesting process and limited cells. Zhu et al. [87] and Zhe et al. [88] assessed the feasibility of ADSC seeded on BAM for bladder reconstruction. The results demonstrated that the approach could promote the regeneration of nervous tissues and smooth muscles in a rabbit model. Another ideal source of cell transplantation involves hUMSC isolated from human umbilical cord tissues. These cells present minimal rejection and do not involve any ethical controversy. Yuan et al. [89] selected hUMSC-seeded BAMs to reconstruct a bladder. The results showed the presence of complete layers of urothelium and smooth muscles.

7. Tissue engineering approaches for urethral reconstruction

Tissue engineering for urethral reconstruction mainly includes the following two approaches: cell-free grafts and cell-seeded grafts. Cell-free matrices are considered as an "off the shelf" material due to the ease of production. Additionally, the morbidity of a donor may be reduced, since no surgical procedures are required for cell harvesting. Table 4 lists the cell-free graft approaches applied in urethral reconstruction in China.

Table 4 Cell-free grafts applied in urethral reconstruction in China.

Research	Animals for modeling	Biomaterial/artificial tissue	Repaired length	Duration of observation
Yang et al., 2004 [90]	Rabbits	Urethral extracellular matrix	1.0—1.5 cm	24 weeks
Wang et al., 2005 [91]	Rabbits	Human cadaveric bladder submucosa	0.5—1.0 cm	24 weeks
Huang et al., 2006 [92]	Rabbits	Porcine SIS	2 cm	12 weeks
Huang et al., 2014 [94]	Rabbits	3D porous BAM	1.5 cm	3 months
Wang et al., 2013 [95]	Rabbit	BAM + PLGA conjugated with VEGF	3 cm	3 months
Lv et al., 2016 [96]	Rabbits	Oxygenating keratin/silk fibroin scaffold	1.5 cm × 0.8 cm	6 months
Jia et al., 2015 [97]	Beagle dog	Collagen-binding VEGF	5 cm	6 months

BAM, bladder acellular matrices; PLGA, polylacticcoglycolic acid; SIS, small intestinal submucosa; VEGF, vascular endothelial growth factor; 3D, three dimensional.

Yang et al. [90] used a urethral ECM from rabbit urethral tissue to repair urethral segmental defects (with a length of 2 cm) in male rabbits. Wang et al. [91] and Huang et al. [92] conducted the similar studies using BAM and SIS to repair urethral segmental defects in rabbits, respectively. Although satisfactory results were obtained in the forementioned studies, the length of the urethra defect is shorter (<2 cm), and the matrices used in urethral reconstruction corresponded to patch grafts and not tubular grafts. Acellular matrices repair urethra by guiding the regeneration of urothelial cells and connective tissue. Thus, an acellular graft may be feasible only when the urethral wall is healthy and rich in blood. A few scaffold parameters, such as porosity, can influence the transport of waste products and nutrients. Appropriate pore size promotes proliferation, infiltration, and differentiation of cells [93]. Huang et al. [94] used BAM treated with peracetic acid (PAA) to form three dimensional structural constructs to repair urethral defects. Their results revealed that PAA-treated BAM promoted the regeneration of urothelium and neovascularization when compared with the non—PAA-treated BAM group. Additionally, VEGF is an important cytokine that promotes angiogenesis, and it plays a major role in urethral reconstruction. Wang et al. [95] suggested BAM stents modified by PLGA with sustained-release of VEGF may serve as a matrix substitute to repair anterior urethral stricture (with a length of 3 cm) in rabbits. In addition to the angiogenesis effect, VEGF also reduces collagen deposition and minimizes scar formation in engineering urethral reconstruction. Lv et al. [96] incorporated an oxygen-generating substance into keratin/silk fibroin to construct oxygen-generative synthetic materials. An evaluation indicated that the oxygen-generative scaffold promoted the regeneration of urinary tract defects and decreased inflammatory response after implantation. The prevalent view is that cell-free biomaterials are feasible in short strictures. Unseeded biomaterials are less successful when the length of urethral strictures exceeds 4 cm [3]. However, Jia et al. [97] utilized collagen scaffolds modified with VEGF to repair extensive urethral defects (with a length of 5 cm) in a beagle dog. Although, biomaterials modified with VEGF may provide a novel strategy for longer urethral reconstruction, it is necessary to conduct further studies to prove the feasibility of this strategy.

To summarize, the applications of cell-free biomaterials in the treatment of urethral strictures are limited. An international consensus is that cell-free biomaterials are only feasible in short strictures when a healthy and well-vascularized part of a urethral wall exists [98]. The limitations of the cell-free grafts prompted researchers to search for other suitable approaches. Autologous cell seeded grafts were proposed to overcome the deficiencies observed with acellular grafts. Multiple studies using cell-seeded grafts in urethral reconstruction are reported in China as listed in Table 5.

Fu et al. [15,99] expanded epidermal cells acquired from autologous foreskin and seeded onto allogeneic rabbit bladder submucosa to reconstruct anterior urethra. The source of epidermal cells is abundant, and the approach of isolating cells is less invasive. Xie et al. [100] selected electrospun silk fibroin matrices as a material and combined it with urothelial cells from bladder biopsies to repair a urethra. This scaffold exhibited 3D properties and porosity, and the poor mechanical properties were improved by post-processing the material. Autologous mesothelial cells isolated from omentum biopsies can be used for tubularized urethral reconstruction. Gu et al. [101] used BAM and mesothelial cells to construct tissue-engineered urethra in a rabbit model.

Furthermore, ADSCs are abundant in quantity and less invasive to a donor site when compared with other cell sources and can differentiate into epithelial cells with the stimulation of factors [102]. Li et al. [38] utilized rabbit adipose-derived stem cells to differentiate epithelium (Epith-rADSCs) and seeded epithelium into BAM to repair urethra defects. Amniotic membrane (AM), which is a urethral substitution with less inflammation and a lower risk of rejection, exhibits significant potential in urethral reconstruction [103]. Wang et al. [104] selected the cell-seeded denuded human amniotic scaffold (dHAS) as urethral reconstruction materials in rabbit models. The evaluation indicated good results in which urethral defects were completely resolved and a mild inflammatory response was observed. Li et al. [105] proposed the reconstruction of tissue-engineered urethra with marrow MSCs and SMCs. Marrow MSCs and SMCs were isolated and constructed into cell sheets prior to seeding into BAM.

It is widely accepted that TGF-β1 may result in scar formation. Li et al. [106] built tissue-engineered urethra with oral keratinocyte and fibroblasts transfected TGF-β1 siRNA. A wide caliber without fibrosis and inflammation was observed after 6 months. Although this approach can

significantly inhibit fibrosis because of the reduction in ECM production, genetic technology limited the application in clinics due to technical aspects. It was demonstrated that TGF-β could promote ECM secretion by regulating the Wnt signaling pathway. Zhang et al. [107] incorporated a Wnt pathway inhibitor (ICG-001) and collagen/poly (L-lactide-co-caprolactone) via an electrospinning technique to construct a scaffold for urethroplasty. A stable oxygen supply is the key point to maintain the survival and cell viability of seeded cells. Huang et al. [108] used a gelatin sponge to create a porous structure for bacterial cellulose scaffolds and selected lingual keratinocytes as seeded cells to repair urethral defects (with a length of 2 cm) in rabbits. The 3D porous structure is conducive to cell infiltration and vascularization, and thus the epithelium layer density and smooth muscle and vessel density were higher.

different from actual clinical urinary tract injuries, and this could account for better results achieved in experimental studies. Furthermore, most researchers in China conducted experiments by using rabbits or rats as opposed to large animals. The urine of rabbits contains high levels of calcium that can penetrate into the collagen due to the strong adsorption capacity [49]. Atala et al. [109] also proposed that large animals can better mimic the conditions prevalent in humans. Although, a large amount of research focused on urinary tract reconstruction was reported in China, clinical studies that limit the application of tissue engineering urethra were not performed. Additionally, a selected ideal cell should be easy to isolate and sufficient in quantity for expansion *in vitro*. Stem cells possess a multiple differentiation potential. Thus, specific environments should be provided for stem cells recruitment and differentiation to regulate migration and directional differentiation [110].

Table 5 Cell-seeded grafts applied in urethral reconstruction in China.

Research	Animals for modeling	Cell types	Biomaterial/artificial tissue	Repaired length	Duration of observation
Fu et al., 2008 [99]	Rabbits	Foreskin epidermal cells	Acellular collagen matrix	1.5 cm	12 months
Fu et al., 2007 [15]	Rabbits	Foreskin epidermal cells	Acellular collagen matrix	1.5 cm	6 months
Xie et al., 2013 [100]	Female beagle dogs	Urothelial cells	Electrospun silk fibroin matrices	3 cm × 1 cm	6 months
Gu et al., 2012 [101]	Rabbits	Mesothelial cells	BAM	1.5 cm	6 months
Li et al., 2013 [105]	Rabbits	BMSCs + SMCs	BAM	2.0 cm	16 weeks
Li et al., 2014 [38]	Rabbits	Epith-rASCs	BAM	2.0 cm × 0.8 cm	6 months
Li et al., 2013 [106]	Rabbits	(TGF-β1 siRNA) fibroblasts + Oral keratinocytes	BAM	2.0 × 0.8 cm	6 months
Wang et al., 2014 [104]	Rabbits	Urethral epithelium cells	Denuded human amniotic scaffold	0.5 cm × 1 cm	3 months
Huang et al., 2015 [108]	Rabbits	Lingual keratinocytes	3D porous bacterial cellulose	2.0 cm × 0.8 cm	3 months
Zhang et al., 2015 [107]	Rabbits	Bladder epithelial cells + fibroblasts	ICG-001 delivering collagen/(PLLA-CL) nanofibrous	2.0 cm × 0.8 cm	3 months

BAM, bladder acellular matrices; BMSCs, bone marrow stem cells; Epith-rADSCs, rabbit adipose-derived stem cells to differentiate epithelium; ICG-001, indocyanine Green-001; PLLA-CL, poly (L-lactide-co-caprolactone); SMCs, smooth muscle cells; TGF-β1, transforming growth factor-β1; 3D, three dimensional.

A cell-seeded graft includes disadvantages such as requiring a period of cell culture in a clean room laboratory. Thus, it is presently not suitable as an "off the shelf" product. There are also cost and time implications. The cost of a cellularized graft is six times that of a non-cellularized graft [3]. It is necessary to perform further studies to reduce the cost-effectiveness of this approach.

8. Current challenges and future directions

Although there is significant progress in urinary tract tissue engineering, it is necessary to resolve certain problems. The creation of an ideal animal model for urinary tract reconstruction is a problem. Most studies created a defect intra-operatively and immediately repaired the same with substitution. However, defects in animal models are

Ideal scaffolds should possess characteristics of biocompatibility, biodegradability, low toxicity, and excellent mechanical strength. It is also important to ensure a suitable microenvironment to promote cell adhesion and tissue organization. Generally, a few synthetic polymers possess excellent mechanical properties and are biodegradable. However, a poor microenvironment exists, and it is similar to the limited application of native tissues in urethral reconstruction. In contrast, naturally derived materials are biocompatible and biodegradable and maintain important bioactive factors. However, these materials possess poor mechanical properties. Fu et al. [71] fabricated composite scaffolds that are constructed synthetic materials combined with a natural matrix by using electrospinning technology. The composite scaffolds favor cell

attachment and distribution due to excellent biocompatibility and mechanical strength. The electrospun composite scaffolds can be used as alternative cell carriers for urinary tract reconstruction. The self-assembly method can construct tissue autologous cells and produce a dense ECM. When compared with other exogenous scaffolds models, the self-assembly method possesses biocompatibility and lower inflammatory response that increases the success rate of urethral reconstruction [111]. In this method, cells can receive differentiation signals in a manner similar to that of native tissues. Although this technique is time consuming, it may provide a promising approach to repair tract tissue defects.

Bioprinting technology has emerged as a new technology for constructing tissue and organs in tissue engineering. This technology enables the simultaneous printing of 3D cells and biomaterials in precise locations within 3D structures. Magnetic resonance imaging and CT scans are used to obtain 3D information related to a tissue or organ and guide specific types of cells and materials into precise locations to mimic the architecture of native tissue construction. Currently, bioprinting techniques can build a series of tissues such as aortic valves [112] and skeletal muscles [113]. Hence, the bioprinting technique exhibits significant potential in tissue engineering and can offer a new approach for tract tissue reconstruction.

9. Conclusion

Although promising progress has been made in the tissue engineering for urinary tract reconstruction in China, these promising results are showed only in animal studies and several issues remain unresolved, such as the ideal cell source, biomaterials and animal models. In addition, further research with larger numbers of patients needs to be conducted to confirm the safety and feasibility of tissue engineering for urinary tract reconstruction. Still, we believe that tissue engineering approach will provide a huge benefit for patients with urinary tract depicts in the future.

Conflicts of interest

The authors declare no conflict of interest.

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