Engineering of the Bladder and Urethra

Xian Lin Yi, Diana Lim, Anthony Atala, and James J. Yoo

Contents

Abstract

Many conditions can damage the lower urinary tract tissues, including trauma, inflammation, cancer, and congenital anomaly. Unfortunately, reconstruction of the human bladder and urethra remains a great urological challenge. This is due to the limited availability of tissue substitutes that can be used for reconstruction.

X. L. Yi
Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Winston Salem, NC, USA

Department of Urology, Tumor Hospital of GuangXi Medical University, Nanning, China

D. Lim · A. Atala · J. J. Yoo (✉)
Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Medical Center Boulevard, Winston-Salem, NC, USA
e-mail: dlim@wakehealth.edu; aatala@wakehealth.edu; jyoo@wakehealth.edu

D. Eberli et al. (eds.), *Organ Tissue Engineering*, Reference Series in Biomedical Engineering, https://doi.org/10.1007/978-3-030-44211-8_1

Consequently, the use of intestinal tissue has remained as the gold standard for bladder reconstruction and repair, despite the associated complications, such as mucus production, electrolyte imbalances, recurrent infections, and malignancies. Similarly, the option for urethral tissue reconstruction is also limited. The autologous buccal mucosa is the most widely used material currently; however, donor site morbidity and stricture recurrence are continued problems. Tissue engineering has been introduced as a promising solution to repair and reconstruct lower urinary tract tissues, including the bladder and urethra. Clinical translation of tissue-engineered products has made significant progress in developing tangible therapies and inspiring the next generation of medical science over recent decades. Cell-based tissue engineering approaches have been employed to treat bladder and urethral pathologies in patients, demonstrating that multicellular tissues and organs with complex functions can be built for clinical use. This chapter covers the recent advancements in tissue engineering in the lower urinary tract tissues. Specifically, we discuss the strategic approaches, components used, supporting technologies, and tissue applications of the bladder and urethra.

1 Introduction

The lower urinary tract is mainly comprised of the bladder and urethra. The bladder is a hollow organ that stores and expels urine through the urethra (Balsara and Li 2017). Anatomically, the bladder is composed of two main parts: a highly specialized urothelium and a compliant detrusor. The bladder wall consists of a urothelial cell-lined lumen surrounded by connective tissue and smooth muscle layers (Ajalloueian et al. 2018). The detrusor muscle, composed of smooth muscle fibers, relaxes to accommodate urine deposition and contracts to expel urine when full. This elastic tissue property permits the storage of a large volume of urine. The urothelium, consisting of transitional epithelial cells, covers the bladder lumen, which acts as a barrier to preventing urine and waste product absorption. The connective tissue layer present between the urothelium and detrusor muscle is lamina propria, which is involved in modulating bladder tissue responses. The lamina propria contains fibroblasts, interstitial cells, and adipocytes, rich in blood vessels, afferent, and efferent nerve endings (Andersson 2014).

The lower portion of the bladder or neck is connected to the urethra, consisting of fibromuscular tubular tissue. The urethra serves as a passage for urine expelled from the bladder and semen from the ejaculatory ducts. The male urethra is 18–20 cm in length and 8–9 mm in diameter and is divided into prostatic, membranous, bulbar, and penile urethra (Partin et al. 2015). The most proximal portion is the prostatic urethra, which commences from the bladder and is entirely enclosed within the prostate. The mid-portion of the prostatic urethra is the opening of the paired ejaculatory ducts. The membranous portion extends from the apex of the prostate to the bulbar urethra. Pseudostratified columnar epithelium covers the membranous urethra and the external urethral sphincter and urogenital diaphragm surrounds it.

From the bulb of the penis to the urethral meatus is the penile urethra (Abbas et al. 2019). In adult females, the urethra is 3–4 cm in length and is embedded behind the symphysis pubis. The urethral wall divided into four layers from innermost to outer are (1) epithelium, (2) sub-mucosa, (3) fascial layer, and (4) two layers of smooth muscles (Gabrich et al. 2007). Squamous epithelial cells line most of the female urethra except for a small group of transitional epithelial cells in the proximal urethra (Liu et al. 2016).

There exist many conditions that can damage the lower urinary tract tissues. These include trauma, inflammation, cancer, and congenital anomaly (Lazzeri et al. 2016). Over the years, reconstructing the lower urinary tract has been performed to repair damaged or diseased tissues using various tissue substitutes, ranging from autologous to allogenic to manufactured synthetic sources. While reconstructive efforts have had variable successes over the years, challenges remain to be addressed on the horizon. Reconstruction of the bladder remains one of the most significant challenges in urology. Since the first cystectomy for bladder cancer in 1887, pursuing the most suitable replacement tissue substitutes for the bladder has been an ongoing challenge (Poole Wilson and Barnard 1971). Consequently, the use of intestinal tissue has remained as the gold standard for bladder reconstruction and repair, despite the associated complications, such as mucus production, electrolyte imbalances, recurrent infections, and malignancies (Adamowicz et al. 2019a; Pi et al. 2018). Similarly, the urethral tissue reconstruction due to trauma or congenital malformation such as hypospadias is also a surgical challenge (Keays and Dave 2017). The autologous buccal mucosa is the most widely used material; however, donor site morbidity and stricture recurrence are continued problems (Caldamone et al. 1998; Markiewicz et al. 2008).

Tissue engineering has been introduced as a promising solution to repair and reconstruct lower urinary tract tissues, including the bladder and urethra. Tissue engineering is a field that applies the principles of cell biology, biomaterials science, and engineering to develop biological substitutes for repairing damaged tissues and restore normal function (Griffith and Naughton 2002; Mangir 2019a). In 2006, the first clinical trial for bladder augmentation using tissue engineering was reported, a milestone in the field (Atala et al. 2006a). Using cell-seeded biodegradable grafts proved that multicellular structure organs with complex functions could be built for clinical use. The urethra is another tissue repaired using tissue engineering techniques in patients with stricture diseases (Raya-Rivera et al. 2011). Clinical translation of tissue-engineered products has made significant progress in developing tangible therapies and inspiring the next generation of medical science over recent decades. Increasing numbers of FDA-approved tissue products are being tested in the clinic, indicating that tissue engineering has become a promising alternative approach for tissue and organ replacement (Griffith and Naughton 2002). This chapter covers the recent advancements in tissue engineering applications in the bladder and urethra. We discuss the strategic approaches, components used, supporting technologies, and tissue applications of the bladder and urethra.

2 Engineering Strategies for Bladder and Urethra

In 1917, the initial application of a free fascia tissue graft for canine bladder augment was reported. Since then, numerous graft materials have been tried in bladder reconstructive studies (Neuhof 1917). In 1950, bladder reconstruction using a plastic mold was performed in dogs (Bohne and Urwiller 1957), and then in humans (Sanchez et al. 1958). The efforts to find ideal materials for bladder tissue reconstruction have been continued. Reconstruction of the human bladder remains one of the greatest challenges in urology. Currently, intestinal tissue is being used as a gold standard for bladder repair. However, because of gastrointestinal tissue incompatibility with urine, a series of complications occur, including mucus production, recurrent urinary tract infections, electrolyte imbalances, and malignancy. As such, investigators have sought to develop alternative methods, including auto-augmentation, tissue expansion, ureterocystoplasty, and regenerative medicine with cell transplantation (Partin 2015).

Regenerative medicine strategies include cell-based therapies, the use of scaffolds seeded with cells, and biomaterials alone (Partin 2015). An ideal neobladder for application in patients should have a large storage capacity, high compliance, low pressure for continence, and voluntary emptying without residual urine (Koie et al. 2018). The desired tissue substitute for urinary bladders' engineering should be nonmigratory, non-antigenic, volume stable, and safe (Partin 2015). In respect of functionalization strategies, bioactive factors could be incorporated into biomaterials and scaffolds. For example, pharmacological factors released by electrospun biomaterials to stimulate new extracellular matrix formation and angiogenesis have been suggested (Mangır et al. 2017; Mangır et al. 2016).

Although the urethra is an adjacent tissue structure connected to the bladder, its engineering approaches depend primarily on the type of tissue substitutes used, such as degradable biomaterials. The ultimate goal is to reconstruct normal functioning urethra that includes the structural and biological properties similar to native tissue. Urethral stricture is a common condition caused by injury, infection, and congenital anomaly, characterized by narrowing or obstruction of urine passage. Management of complicated, lengthy urethral strictures is considered a difficult and challenging task due to frequent recurrence rates. Many tissue substitutes have been used for urethral repair applications, such as skin and oral mucosa grafts; however, these materials have not been entirely satisfactory (Mangir 2019a). Recent success in human urethra repair using tissue engineering techniques provides hopes to patients in need (Raya-Rivera et al. 2011).

Urethral tissue engineering strategies require the universal requirements such as proper mechanical compliance, barrier function to avoid urine leakage to the underlying layer, and specific considerations for different urethral segments such as elastic properties to accommodate structural changes during the erection of the penis. Furthermore, the engineered tissue compliance is vital in adapting pressure during micturition, where the bladder pressure increases up to 50–60 cm H_2O with the urine flow rate of approximately 20–30 mL/s (Mijailovich et al. 2007). Therefore, urethral tissue with passive viscoelastic properties must prevent the escalation of pressure in

the bladder that could reflux and damage the upper urinary tract (Yalla and Burros 1974). It is known that even a very short stricture can affect renal function. The most common cause of renal transplantation before puberty is posterior urethral valves in children where normal urine flow is disrupted (Husmann and Rathbun 2006).

To achieve urethral tissue's functional properties, efforts have focused on developing tissue constructs that replicate the native tissue configuration, consisting of smooth muscle and epithelial layers. The epithelial lining is an important tissue component that prevents leakage of cytotoxic urine into the body, thus avoiding the possible occurrence of inflammation, fibrosis, and stricture (Akkad 2007). For the cell-based tissue constructs, the viability of cells within the scaffold can be affected by exposure to urine (Rajasekaran et al. 2006). Therefore, an indwelling catheter is usually placed after surgery to divert urine to provide adequate time for the cells to mature and cover the urethral lumen to perform the urothelial barrier function. *The* cell sheet engineering technique, which allows for cellular sheet fabrication, has been applied (Kajbafzadeh et al. 2017).

3 Vascularization

Establishing a vascular network is critical in achieving successful tissue engineering therapies. Vascularization involves the sprouting of capillaries from existing blood vessels in vivo. This process involves complex interactions among endothelial and non-endothelial cells, growth factors, enzymes, and adhesion molecules. Many approaches have been introduced to promote vascularization, including the use of biological factors, such as growth factors and endothelial cells to stimulate angiogenesis. Other strategies include cell sheet engineering, scaffold functionalization, arteriovenous loops, spheroid coculture, modular assembly, and bioprinting. Toward this goal, one study designed a scaffold that promotes attachment and proliferation of the endothelial cell, thus facilitating the capillary formation. The addition of growth factors into the microenvironment and incorporating a modified angiogenic cell source was also attempted (Mangir et al. 2019b). Recently, a phase 2 clinical study of a bioengineered human acellular vessel (HAV) was performed and evaluated. A biodegradable mesh scaffold seeded with human vascular cells was implanted as a hemodialysis conduit in patients with end-stage renal disease. After 16 to 200 weeks follow-up, human acellular vessels were transformed into functional multilayered living tissues by endothelial, myogenic, and progenitor cells of the host. The new vessels were functional in transporting blood and self-healing after cannulation injury (Dahl et al. 2011a). While this approach may not directly apply to vascularize implanted tissues, it is hopeful that this technology may be modified and further developed to benefit tissue implants in the future. Attempts to enhance vascularization have been tried in bladder urethral tissue applications. Rapid angiogenesis is crucial for tissue regeneration, particularly for large-sized grafts. Overcoming the diffusion limitation is key to success, and numerous investigators have sought to solve this challenge (Rouwkema et al. 2008).

Establishing vascularization of full-thickness engineered soft tissue constructs with multilayer epithelium is not easily achieved, leading to cell necrosis and shedding. Shukui Zhou et al. have monitored the tissue-engineered urethra construct after subcutaneous implantation (Zhou et al. 2017). The investigators observed that new capillaries infiltrated into the implant and maintained an intact epithelial layer. However, blood capillaries were not fully matured. This study indicates that the implanted tissue can recruit new blood vessels that support the survival of cells. Another study used a rat-tail collagen-based acellular collagen scaffold to repair urethral defects in rabbits (Pinnagoda et al. 2016). Repopulation of urothelial and muscle cells was observed on all grafts with vessel formation at 1 month. To further facilitate vascularization, one group conjugated vascular endothelial growth factor (VEGF) to collagen (Jia et al. 2015). In this study, a modified tubularized collagen scaffold was used to repair a 5-cm long anterior urethral defect in dogs. The results demonstrated that the thickness of the epithelial layers was maintained with increased formation of blood vessels in the VEGF conjugated scaffold group.

4 Bioreactors

Bioreactors have become a standard supporting technology for tissue engineering. They serve many functions, from tissue preconditioning and maturation of tissue constructs to large-scale cell cultures (Martin et al. 2004). Bioreactors are complex engineering simulation biosystems that can control environmental factors (pH, dO_2, and T°) to recapitulate physiological body conditions (de Bournonville et al. 2018). Bioreactor systems have been used for bladder and urethral tissue applications. For example, organization and maturation of engineered bladder tissue construct can be simulated by providing mechanical filling and emptying control using an in vitro bioreactor (Haberstroh et al. 2002). In vivo bioreactors can improve vascularization of the bioengineered bladder, decrease the loss of contractility, and prevent fibrosis (Horst et al. 2013a).

Precise mechanical and nutrient environmental controls are also crucial in bladder bioreactors for enhancing cell proliferation and differentiation (Farhat and Yeger 2008). The goal is to achieve an impermeable epithelial surface, well-differentiated urothelial and smooth muscle cells, and excellent compliant extracellular matrices (Serrano-Aroca et al. 2018a). It is reported that mechanical stimulations positively affect the growth and development of newly developed tissues; however, high shear stress inhibits the proliferation of endothelial and smooth muscle cells (Stock and Vacanti 2001). In one study, Faisal M. Shaikh et al. have developed a pulsatile bioreactor that exerts dynamic uniform cyclical pressure over a urinary bladder matrix (UBM) scaffold (Shaikh et al. 2010). When cyclical mechanical pressure was applied on a cell-seeded urinary bladder matrix scaffold, significantly greater growth and urothelium viability were observed in the bioreactor compared with the conventional static conditions. A commercialized bioreactor system (BOSE BioDynamic®) was applied to urothelial and smooth muscle cells seeded collagen scaffolds. Mechanical stimulation of smooth muscle cells promoted cell growth,

improved cell alignment, and distribution. However, dynamic mechanical stimulation did not affect the proliferation and differentiation of urothelial cells.

Mechanical stimulation in urethral engineering is beneficial in maturing the engineered tissue constructs. Magnan et al. investigated the self-assembly technique for urethral tissue constructs using an in vitro bioreactor (Magnan et al. 2009). Their results showed that the engineered tissue grafts displayed sufficient resistance to the pressure applied by the bioreactor. Cattan et al. have combined the self-assembled scaffold with mechanical stimulation with in vitro bioreactors to produce tissue-engineered tubular graft (Cattan et al. 2011). Their results showed that mechanical stimulation leads to maturation and formation of a stratified urothelium.

5 Biomaterials for Bladder and Urethra

Biomaterials are a major component of tissue engineering. Ideal biomaterials for scaffold should be biocompatible, non-antigenic, and biodegradable. The scaffolds provide structural support and a suitable microenvironment for cells to attach and proliferate (Chan and Leong 2008). Therefore, scaffolds should be designed and fabricated according to the specifics of tissue being engineered. In this regard, the bladder scaffold should provide storage of adequate urine volume and the ability to contract for physiologic voiding. Thus, many tissues and biomaterials of diverse nature have been examined for tissue engineering of the bladder. The bladder scaffold made by natural or synthetic biomaterials is the key element supporting the bladder function (Oberpenning et al. 1999a). The scaffold for the bladder should endure dynamic mechanical and chemical stimuli under normal physiological filling and emptying. The proliferation, migration, and differentiation of the seeded cells will be affected by the matrix (Oberpenning et al. 1999a). Therefore, the biomaterials used for bladder applications should be biocompatible and possess sufficient mechanical and chemical properties. Furthermore, the luminal surface should allow for uniform urothelial cell attachment to prevent urine leakage into the sub-urothelial tissue, and the outer side should harbor the smooth muscle cells for contraction, innervation, and vascularization (Horst et al. 2013b).

Similar to the scaffolds for bladder, the ideal biomaterial for the urethra should possess adequate mechanical support to prevent premature collapse before tissue regeneration is achieved (Orabi et al. 2013a). One study shows that a self-assembled engineered cell sheet has many of these favorable characteristics. It is reported that a well-stratified urothelial cell layer formed on self-assembled collagen sheets can withstand a high burst pressure in a porcine model (Bouhout et al. 2010). The self-assembled collagen sheets made from human adipose-derived stromal cells also showed similar mechanical and architectural characteristics (Rousseau et al. 2015). Nondegradable materials were also considered for urinary tract applications; however, they resulted in calcification, chronic hematuria, stone formation, fistulae, and graft contracture (Bouhout et al. 2010; Feil et al. 2006). Degradable biopolymers, on the other hand, allowed for tissue ingrowth, resulting in becoming tissues closer to the native normal urethra.

Degradable polymers for urethral tissue engineering stem from natural sources or by synthesis. Natural biomaterials such as acellular matrices and natural polymers are known to promote cell growth and differentiation and enhance angiogenesis (Ouellet et al. 2011). Currently, the most widely used polymers for urinary tract tissue applications include linear polyesters, copolymers, poly-lactic acid (PLA), poly-glycolic acid (PGA), poly lactic-co-glycolide (PLGA), and polycaprolactone (PCL). Synthetic polymers have the advantage of providing reproducible mechanical characteristics and controlled degradation.

6 Decellularized Matrices

Two classes of biomaterials, acellular matrices(Chen et al. 1999a) and biopolymers (Oberpenning et al. 1999a), have been used as scaffolds in bladder regeneration. These biomaterials can be configured as three-dimensional porous structures (Dhandayuthapani et al. 2011a), in which the seeded scaffold construct can serve as a template for the growth of new tissue (Ajalloueian et al. 2018). Tissue-derived decellularized matrices have been favorably used for bladder and urethra applications due to their biocompatibility and similarity in composition and inner architecture to native tissue (Ahmed 2019). Allogenic acellular matrices, such as the bladder submucosa matrix, offer excellent trophic support, thus stimulating tissue growth, facilitating cell–material interactions, maintaining the functional phenotype (Chun et al. 2007). Decellularized bladder tissue-derived scaffold is usually obtained from porcine due to its similarity to human bladders in anatomy and biology (Crapo et al. 2011; Zhang et al. 2000). It has been shown that the decellularized matrix facilitates cellular adhesion, proliferation, and maturation. However, some disadvantages include potential process-induced damage to the extracellular matrix ultrastructure and mechanical properties. In addition, the host innate immune response may be induced by the degradation of extracellular matrix scaffolds (Taylor et al. 2018).

Extracellular spaces contain a network of extracellular matrix (ECM) proteins and polysaccharides (Alberts et al. 2002). The ECM is an intricate crosslinked network structure of proteins, growth factors, small molecules, and glycosaminoglycans, and the commonly used tissue ECM for urinary tract applications include bladder acellular matrix (BAM), small intestinal submucosa (SIS), pericardium, fascia lata, amniotic membrane, and tunica vaginalis (Ramuta and Kreft 2018). ECM's main constituents are collagens, fibronectin, elastin, laminins, proteoglycans, glycoproteins, and glycosaminoglycans (Theocharis et al. 2016). As an example, an ECM scaffold was generated by cells seeded on polyglycolic acid scaffolds to deposit ECM, followed by a decellularization process to produce a vascular graft. The FDA approved this product for clinical use (Dahl et al. 2011b; Niklason et al. 1999). The acellular vascular graft or human acellular vessel (HAV) maintained mechanical integrity when implanted in patients after up to 4 years follow-up (Kirkton et al. 2019). In one study involving urethral repair, ECM scaffolds were shown to play a signaling role and maintained cellular homeostasis. More importantly, ECM is found to regulate the urethra's mechanical compliance of the urethra by dilating its lumen

during micturition and stretch during erections (Shirozu et al. 1995; Ohel et al. 1995).

The typical and frequently used decellularized xenogeneic or allogeneic matrices are porcine small intestine submucosa (SIS) and human bladder acellular matrix (BAM). As a collagen-based biomaterial, SIS is processed by mechanically removing the mucosal, muscular, and serosal layers from the inner and outer surfaces, yielding a collagen-rich membrane of approximately 0.15–0.25 mm in thickness, mostly consisting of the submucosal tissue (Badylak et al. 1989). In the 1980s, Badylak et al. first applied SIS for bladder augmentation in dogs (Badylak et al. 1989). In 2005, Lu et al. (Lu et al. 2005) seeded cells onto SIS scaffolds. In their study, histological results indicated that seeded muscle-derived cells migrated throughout the graft after 20 days, and initial remodeling of the SIS occurred within 10 days. However, moderate-to-heavy adhesion and graft shrinkage was reported in both the cell-seeded and unseeded SIS by Zhang and colleagues (Zhang et al. 2006a). Many studies have demonstrated that the urothelium regeneration was achieved quicker than the muscle layer (Kropp et al. 1996; Caione et al. 2012). In another report, Schaefer et al. suggested that unseeded small intestine submucosa is not well suited for human enterocystoplasty because of the insufficient increase in bladder compliance (Schaefer et al. 2013). In other studies, SIS was shown to promote cellular growth and angiogenesis. The results of SIS was comparable to skin and mucosal grafts in animal experiments of urethral replacement (Fiala et al. 2007; Palminteri et al. 2012). SIS also showed favorable outcomes when used as a corporal body graft (Hayn et al. 2009) and as a seeded graft in a urethroplasty (Zhang et al. 2016). It was found that SIS decreased the risk of urine extravasation and reduced early irritation when used for urethral replacement (Yoo et al. 1998). However, the data of clinical experiments with SIS were not entirely satisfactory, mainly caused by infection (Orabi et al. 2013b).

Bladder acellular matrix (BAM) is an attractive biomaterial for tissue engineering (Zhang et al. 2006b). It is an ECM with an intact microarchitecture, first described in 1975 (Brown et al. 2005). One of the first applications of BAM was to support the regeneration of the rat bladder wall (Probst et al. 1997). A study evaluated the potential of the bladder submucosa matrix for dog bladder augmentation (Yoo et al. 1998). In this study, one side of the scaffold was seeded with autologous urothelial cells and the opposite side with smooth muscle cells followed by augmentation cystoplasty. The result showed that a 99% increase in capacity was observed in the cell-seeded group, whereas the unseeded group showed only a 30% increase. This study concluded that cell-seeded allogenic bladder submucosa is an excellent biomaterial for bladder augmentation. This research paved the way for the subsequent human bladder augmentations (Atala et al. 2006b). In another study, BAM seeded with marrow-derived mesenchymal stem cells was transplanted in rats after partial cystectomy. Histological analyses showed increased muscle regeneration and the bladder capacity nearly recovered completely for up to 6 months (Coutu et al. 2014).

BAM and SIS have been widely used for urethral tissue repair experimentally and clinically (Davis et al. 2018a). In a rabbit model of urethroplasty, cell seeded BAM

scaffold demonstrated a normal urethral architecture when implanted for 4 weeks, and achieved near natural urethra after 6 months (Chun et al. 2015; Gu et al. 2012). In another rabbit study, autologous urethral tissue was repaired with combined BAM and suggested that the use of BAM may be a viable option for long-segment urethral stricture (Chun et al. 2015). However, residual immunogenic components are a safety concern of BAM, despite the stringent decellularization process (Roth and Kropp 2009). Other tissue-derived scaffolds have been developed for urinary tract applications. In one study, decellularized trachea was applied as a tissue-engineered neo-urinary conduit (Anirudha et al. 2018). In this study, a decellularized rabbit trachea was seeded with human smooth muscle cells and urothelial cells. The results showed that decellularized trachea possesses appropriate biomechanical properties and structural integrity. Another promising natural scaffold biomaterial proposed for bladder applications is tissue-engineered pericardium, which has achieved favorable results in the test of small-sized scaffolds (Kajbafzadeh et al. 2011).

Tissue-derived matrices are biocompatible, durable, effective, and are easily remodeled (Horst et al. 2013c). However, seeding smooth muscle cells is difficult to infiltrate into the scaffold because of the high density of these materials (Atala 2011). They are also prone to fibrosis and exhibit poor mechanical and dimensional stability. Therefore, artificial tissue-engineered biomaterials or a combination of synthetic and biological materials may be a choice for use in bladder reconstructive surgery (Zhang et al. 2006b).

7 Natural Polymers

Natural polymers have been frequently used for many tissue engineering studies due to their biocompatibility, less toxicity, and superior interactions with cells compared with synthetic polymers (Dhandayuthapani et al. 2011b). Collagen, gelatin, fibrinogen, and elastin are more commonly used scaffold materials for bladder and urethra applications. Collagen is an ECM-derived natural material and plays a crucial role in promoting cell attachment and maintaining structural integrity, thus improves tissue regeneration (Hubbell 2003) and decreases inflammatory and antigenic responses (Furthmayr and Timpl 1976). Many collagen-based materials have been tried for urethral reconstruction such as collagen gels, bladder-derived acellular submucosa and acellular urethral submucosa (Partin et al. 2015). In one study, rat-tail collagen was used to generate acellular collagen tubular scaffolds to repair a 2 cm-long rabbit urethral defect (Pinnagoda et al. 2016). The collagen tube possessed good surgical handling and did not require synthetic polymer support to maintain structural integrity. Results demonstrated that spontaneous repopulation of urothelial and muscle cells was observed on all grafts. The cellular organization increased with time, although fistula and stenosis occurred in 20% of the animals postoperatively.

As a natural polymer, silk fibroin (SF) is derived from Bombyx mori cocoons. SF is a biocompatible polymer that can degrade into peptides and amino acids (Wang et al. 2008). SF has been used for urethral applications. In one study, urothelial cell-seeded silk fibroin grafts were functioning without causing strictures after implanted

for 6 months in urethroplasty (Xie et al. 2013). Bilayer silk fibroin (BLSF) scaffolds have also been evaluated for urethral repair in a rabbit model of onlay urethroplasty. Histological analyses demonstrated the formation of innervated and vascularized neotissues at graft sites (Algarrahi et al. 2018). The bilayer silk fibroin (BLSF) matrices were recently investigated in pigs with acute partial bladder outlet obstruction. The animals underwent augmentation cystoplasty with BLSF grafts. The bladder capacity and compliance in the graft augmented group significantly increased by 79% and 171%, respectively, compared to the baseline values after 3 months. The study shows that BLSF scaffolds can improve the capacity and compliance of bladder and promote the formation of neotissues (Saif et al. 2019). Another important natural polymer is hyaluronic acid (HA), which has been applied in wound healing and 3D bioprinting applications (Burdick and Prestwich 2011). The US Food and Drug Administration (FDA) also approved another natural polymer, alginate, used for bladder tissue engineering after modification with cell-adherent peptides (Lee and Mooney 2012).

8 Synthetic Biomaterials

Over the past decades, many synthetic and organic materials have been used for bladder regeneration. Synthetic materials used for bladder and urethral tissue applications include polyvinyl sponge, Teflon, collagen matrices, polylactic acid (PLA), poly (glycolic acid) (PGA), Polycaprolactone (PCL) (Pinnagoda et al. 2016), copoly(lactic/glycolic) acid (PLGA) (Yao et al. 2013; El-Taji et al. 2015), poly(ε-caprolactone)/poly (L-lactic acid) (PCL/PLLA)(Shakhssalim et al. 2013), Poly (Carbonate-Urethane) Urea (PCUU), Nanocellulose (Bacakova et al. 2019), poly(1,8-octanediol-co-cirtic acid) (POC)(Liu et al. 2016), and silicone (Serrano-Aroca et al. 2018b). These polymers can be fabricated reproducibly, with controllable mechanical and degradation properties, porosity, and structures (Horst et al. 2013c). Electrospun nanofibers with core-shell structure have been applied in rat bladder augmentation. Domed scaffold fabricated by coaxial electrospinning of poly(l-lactide)/poly(e-caprolactone) (PLCL) and Hyaluronic acid (HA) mesh possessed good cytocompatibility and was convenient for smooth muscle tissue proliferation (Chunxiang et al. 2019). In another study, Poly (Carbonate-Urethane) Urea (PCUU) scaffold for bladder engineering was evaluated. The biodegradable elastomers showed cytocompatibility, increased porosity, and stretch, which can aid smooth muscle cells infiltration. Bladder augmentation with Poly (Carbonate-Urethane) Urea scaffolds can increase the bladder capacity and voiding volume, and enhance survival of the rat disease model (Sivaraman et al. 2019). Synthetic matrices seeded with cells have been applied for urethral reconstruction. A widely used synthetic material for urethral repair is polylactide-co-glycolide (PLGA) today (Adamowicz et al. 2019b).

While there are advantages to using synthetic materials, the disadvantages include urinary stone formation and mechanical failure for nondegradable polymers. In contrast, degradable materials often result in fibrosis, scarring, graft contracture, and a long-term reduction of voiding volume (Partin 2015). Other disadvantages of

synthetic materials are their biological inertness, foreign body reaction, the lack of trophic support for cell activity, and the natural barrier function of urothelium (Horst et al. 2019). It has been reported that pre-seeding the scaffolds with cells is shown to relieve graft shrinkage (Zhang et al. 2006b; Filippo et al. 2002). However, this process requires time-consuming preparatory steps of cell harvesting, isolation, seeding, and proliferation on scaffolds. Despite the laborious procedures, the preferred option for bladder engineering seems to be the use of autologous cells in combination with synthetic biomaterials (Horst et al. 2019).

To be able to use synthetic polymers for cell-based applications, the surface of polymers must be in favor of cell attachment. A novel surface-modification of poly-L-lactic acid (PLLA) scaffold was produced to support urethral cell adhesion and proliferation (Fu et al. 2011). In this study, a hollow cylinder was wrapped with PLLA filaments, followed by chitosan and fibronectin treatment. Rabbit uroepithelial cells were adhered to and cultivated on the scaffold. The scaffold has more than 90% porosity, and its mesh was fully populated by uroepithelial cells after 3–7 days. In another study, electrospun Poly(l-lactide)/Poly(ethylene glycol) (PLLA/PEG) hybrid scaffolds were produced to support urethral tissue regeneration (Lv et al. 2016). The scaffolds were then seeded with human amniotic mesenchymal cells (hAMSCs) for repairing rabbit urethral defects. The results showed that hAMSCs-loaded PLLA/PEG scaffolds produced multilayered urothelium with similar characteristics to the native urethra.

Cellulose is known to be the most abundant biopolymer on earth. Nanocellulose is the cellulose of nanostructures with a feature that, at least in one dimension, is not exceeding 100 nm (Bacakova et al. 2019). A three-dimensional porous bacterial cellulose (BC) scaffold seeded with lingual keratinocytes was used to repair rabbit ventral urethral defects. The results demonstrated that all urethras maintained a wide caliber in 3D porous BC seeded with cells. The smooth muscle content and endothelia density were significantly increased at 3 months. This study shows that the BC scaffolds seeded with cells can enhance urethral tissue regeneration (Huang et al. 2015).

9 Smart and Hybrid Polymers

Smart biomimetic materials are a significant advancement in the field of tissue engineering. Some smart biomaterials are designed to respond reversibly to temperature, pH scale, light, or ionic strength (Aguilar and San Román 2019). The responses of these polymers may include gelation, reversible adsorption on a surface, collapse of a hydrogel, and alteration between hydrophilic and hydrophobic states. Smart biomaterials have been studied in many areas, including drug delivery, medical devices, and tissue engineering applications (Kowalski et al. 2018). Furthermore, combinations of natural and synthetic polymers can be designed to produce hybrid biomaterials with specific properties for tissue engineering. Such properties may include controlling porosity, mechanical strength, cell affinity, biocompatibility, and biodegradability (Chun et al. 2015).

A group of electroconductive polymers has demonstrated promise for engineering electrically active tissues. Electroactive polyurethane polymers have manifested enormous potential in bladder tissue engineering, especially in regenerating muscular components and innervation (Hardy et al. 2015; Wu et al. 2016). Electroconductive polymers may be applied with electrical stimulation for advanced maturation of engineered urethra (StÖlting et al. 2016). In one study, a smart acellular collagen scaffold with growth factors was used in sheep bladder regeneration. The results showed that the presence of growth factors, VEGF, FGF2, and HB-EGF improved bladder regeneration (Roelofs et al. 2016).

The largest class of smart polymers used is thermo-responsive polymers. A reversibly alterable phase or volume transition can occur following a change of temperature in these materials. A typically used biomaterial for this purpose is poly (N-isopropylacrylamide) (PNIPAM). The lower critical solution temperature (LCST) of PNIPAM is around 32 °C, not far from the human body temperature (Kim and Matsunaga 2017). Accordingly, PNIPAM solution can be changed from a hydrophobic to hydrophilic surface following the change of temperature in water. When the temperature is above the lower critical solution temperature, the polymer chains of PNIPAM are precipitated, making it hydrophobic, while below LCST, polymer chains hydrated and made PNIPAM hydrophilic (Kim and Matsunaga 2017). This characteristic is beneficial in producing three-layered urethral tissue constructs. It was reported that viable urothelial cell sheets could be generated using the temperature-responsive cell culture method (Shiroyanagi et al. 2003).

A smart bilayered scaffold seeded with keratinocytes and muscle cells was also tested in canine urethral reconstruction. The bilayer scaffold consisted of a microporous network of silk fibroin (SF) and a nanoporous bacterial cellulose (BC) scaffold with a porosity of 85%. The result showed that the smart SF-BC structures could enhance adhesion and proliferation of lingual keratinocytes and lingual muscle cells. At 3 months after graft implantation, the urethra reconstructed with the seeded SF-BC scaffold showed superior structure formation compared to the unseeded control scaffold. The nanoporous network offers excellent support for epithelial cells, while the microporous scaffolds sustain the growth and penetration of smooth muscle cells (Lv et al. 2018). Shape-memory polymers (SMPs) have also been tested in vascular and bone tissue engineering. The characteristics of SMPs are suitable for urethral tissue engineering, as urethra is subject to expansion during erection and recoils during the flaccid state. Smart acellular collagen scaffold with growth factors have been applied in sheep bladder regeneration studies (Roelofs et al. 2016).

10 Cells for Engineering Bladder and Urethra

Cells are an integral part of tissue engineering. While cells from various sources have been used for tissue regeneration studies, autologous cells are considered the preferred source for bladder and urethral applications in patients currently (Rashidbenam et al. 2019). Experimentally, both somatic and stem cells have been

used for bladder and urethra tissue studies, spanning from autologous, allogeneic, and xenogeneic sources.

10.1 Somatic Cells

One tissue engineering strategy that has been shown to be effective in reconstructing bladder and urethra tissues involves the use of autologous cells from the bladder (Zhang and Frey 2003). The donor tissue is dissociated to isolate urothelial and muscle cells. The cells are then culture expanded, attached to a supporting matrix followed by implantation back into the same host (Atala et al. 1992; Li et al. 2008; Feng et al. 2011; Atala 2009). This approach has shown to provide sufficient numbers of cells for bladder and urethral tissue applications clinically. While the ideal cell source for tissue application is from the target organ of interest, there are instances where obtaining a tissue biopsy from the host may not be feasible. This is true when the organ or tissue to be reconstructed cannot provide healthy cells, such as bladder cancer. In such a case, cells, either somatic or stem cells, should be obtained from other tissue sources.

Epithelial cells for urethral tissue engineering could originate from different organs, such as the bladder, penile foreskin, and buccal mucosa. In addition, autologous urothelial cells can also be isolated from urine (Nagele et al. 2008). In one study, the penile foreskin was used as a source of autologous epidermal cells. These cells were seeded on acellular collagen matrix and used as a tissue implant for urethral applications in animal models with positive results (Fu et al. 2007). Autologous oral keratinocytes was previously obtained from a buccal mucosa biopsy for urethroplasty (Barbagli et al. 2018). The sources for smooth muscle cells include the bladder, blood vessel, and gastrointestinal tissues. The culture of both cell types is well established and their expansion capacity far exceeds the cell numbers necessary for reconstruction.

10.2 Stem Cells

Stem cells have become an attractive cell source for reconstructive tissue engineering due to their ability to differentiate into multiple cell types, and if guided appropriately, they can transform into target cells of interest. For this reason, stem cells have been used extensively in urinary tract application studies (Serrano-Aroca et al. 2018a). One stem cell type that is widely investigated is adult mesenchymal stem cells (MSCs), which can be isolated from multiple body sources such as bone marrow and adipose tissue (Adipose-derived Stem cells; ASCs). Mesenchymal stem cells have been shown to be safe and effective in many tissue applications, including the urethra (Stangel-Wojcikiewicz et al. 2014; Deng et al. 2014). Moreover, these cells possess immunomodulatory properties, thus eliminating the need for autologous cells for tissue applications (Davis et al. 2018b).

Adipose-derived stem cells have been widely used due to the ease of harvest, isolation, and expansion as compared to other sources of MSCs. They are also immunomodulatory and can secrete various growth factors to promote angiogenesis and neurogenesis (Kingham et al. 2014). As such, ASCs have been favored as a cell source for bladder regeneration studies (Wang et al. 2019). These cells have been shown to differentiate into smooth muscle (Salem et al. 2013) and urothelial-like phenotype (Zhang et al. 2013), thus avoiding the use of native cells from bladder cancer patients. In one study, bioengineered bladder patches constructed from multilayered adipose-derived stem cell sheets were successfully used for bladder regeneration (Wang et al. 2019). The effectiveness of ASCs has also been demonstrated in urethral applications. Davis NF et al. have reviewed 11 in vivo preclinical studies that investigated various stem-cell therapies for reconstructing the urethra (Davis et al. 2018b). Among them, ten studies showed the data on the patency in the stem cell-seeded bio-scaffolds; 100% patency was reported in nine studies, and the remaining one was 75% patency rate. These studies indicate that stem cell-seeded urethral scaffolds can recruit endogenous cells for tissue regeneration, and this effect is believed to be moderated by angiogenic trophic factors via a paracrine mechanism of stem cells (Caplan and Dennis 2006). In another study, Zhou S et al. used the myoblast-induced adipose-derived stem cell sheets to form muscular layers (Zhou et al. 2017). The adipose-derived stem cells were isolated from fat tissue in the groin of beagle dogs. The cells were cultured in myoblast differentiated culture medium and then stimulated with vitamin C to form ECM. The structure and function of neo-urethras were similar to normal urethra after 3 months. Human ASCs were also used with self-assembled collagen sheets. The mechanical and architectural characteristics were shown to be similar to that of human fibroblasts (Rousseau et al. 2015).

It has been demonstrated that bone marrow-derived stem cells (BMSCs) have the potential to differentiate into bladder muscle (Shukla et al. 2008a) and urothelial cells (Anumanthan et al. 2008). The differentiated cells resembled bladder smooth muscle cells and promoted muscle bundle formation (Zhang et al. 2005). A study used the bladder acellular matrix seeded with BMSCs for bladder tissue engineering. The results showed that the BMSC-seeded bladder acellular matrix improved nearly 100% of normal bladder capacity for up to 6 months after partial cystectomy (Coutu et al. 2014). In another study, a research team performed bladder augmentation after hemicystectomy in six dogs using the BMSC-seeded small intestine submucosa (SIS) (Zhang et al. 2005). Upon retrieval, solid smooth muscle bundle formation was observed throughout the cell-seeded grafts. In the unseeded SIS scaffolds, SMC regeneration was seen only at the graft edges adjacent to native tissue, suggesting the effects of BMSCs. In a porcine model, Shukla and colleagues also showed that the BMSCs were able to differentiate into smooth muscle in the pig (Shukla et al. 2008b). Recently, bladder tissue consisting of full three layers was successfully generated using differentiated human BMSCs (Gabr et al. 2018). BMSCs were cocultured separately with urothelial and muscle cell-derived conditioned medium to obtain the corresponding biological characterization. These cells were then seeded on the decellularized bladder adventitia scaffold. Histological examination of the recellularized tissue revealed the full three layers of bladder tissue (Shukla et al.

2008b). The advantage of synergistic effect of allogeneic BMSCs and endothelial progenitor cells (EPCs) was also investigated for treatment in a long segment urethral defect. These cells were seeded onto decellularized human amniotic scaffolds (dHAS) to repair a 3 cm-long circumferential urethral defect in dogs. The animals showed unhindered urination and capacious urethral caliber similar to the normal group in dHAS+BMSCs+EPCs and dHAS+EPCs groups (Chen et al. 2018).

Induced pluripotent stem cells (iPSCs), generated by somatic cell reprograming, have recently gained enormous attention as a non-controversial cell source of embryonic stem cells (Takahashi and Yamanaka 2006). These cells possess self-renewal properties and the ability to differentiate into various specialized cell types (Brivanlou et al. 2003). Due to the enormous potential of utility, iPSCs have been extensively investigated in numerous areas. Likewise, iPSCs have been studied in urology. These cells have been shown to induce and differentiate into urothelial cells and smooth muscle cells (Osborn et al. 2014; Zhe et al. 2016). However, further studies are necessary to use iPSCs for tissue applications.

Amniotic fluid and placenta stem cells are fetal-derived cells that have great potential for development. These cells do not present with ethical controversies that embryonic stem cells have but exhibit similar cellular characteristics. The cells are multipotent, highly replicative, and can differentiate into all three germ layers (De Coppi et al. 2007a; De Coppi et al. 2007b). These cells have been studied in many cell-based applications. In one study, human amniotic mesenchymal cells (hAMSCs) were seeded on Poly(l-lactide)/Poly(ethylene glycol) scaffolds and implanted to repair urethral defects in rabbits. The hAMSCs-seeded scaffold showed that epithelial cells covered the defect and formed multilayer mucosa membranes similar to normal urethra after implanted for 12 weeks (Lv et al. 2016).

Another stem cell type gaining much attention in urology is urine-derived stem cells (USCs) due to their existence in urine (Zhang et al. 2008). These cells are found to possess stem cell characteristics, robust proliferative, and differentiation potential. More importantly, these cells are easily obtained from voided urine, and the isolation procedures are simple and low costing. USCs have been shown to differentiate into many cell types, including urothelial, smooth muscle, vascular endothelial, osteogenic, neurogenic, and adipogenic cells (Bharadwaj et al. 2011; Bodin et al. 2010). USCs have been used in urological cell-based applications for urethral and bladder reconstructions (Bodin et al. 2010; Wu et al. 2011; Bharadwaj et al. 2013).

11 Applications of Bladder and Urethra

For more than 100 years, gastrointestinal segments have been used for bladder replacement or repair in the clinic, despite the associated complications (Partin 2015; Anderson and McKiernan 2018). Tissue engineering approaches were initially applied to address the reconstructive challenges associated with pathologic bladder and urethra conditions. Early studies have tested different scaffolds as cell carriers. PGA scaffold coated with PLGA were examined to determine whether the synthetic biomaterial could serve as a comparable biomaterial as acellular scaffolds. Human

bladder urothelial and muscle cells were attached to both collagen matrices derived for bladder submucosa and nonwoven meshes of PGA in vitro (Atala et al. 1993; Cilento et al. 1994). When implanted into animals, these constructs were shown to survive and reorganize into newly formed multilayered structures that exhibit spatial orientation and vascularized in vivo. In a subsequent study, autologous urothelial and smooth muscle cells were seeded on PGA scaffold, and implanted the tissue-engineered neobladder in a canine subtotal cystectomy model. The tissue-engineered neo-organs, consisting of a bladder-shaped biodegradable polymer and autologous urothelial and muscle cells showed a normal bladder capacity and compliance and histologic findings comparable to normal bladder tissue. This was the first report demonstrating the successful reconstruction of the tissue-engineered bladder in a large animal model (Oberpenning et al. 1999b).

The successful preclinical study led to a clinical study using the tissue-engineered bladder construct. Autologous tissue-engineered bladder substitutes were used to treat patients with poorly compliant neurogenic bladders due to congenital anomaly (Atala et al. 2006a). The scaffolds made of collagen and polyglycolic acid (PGA) were fabricated for augmentation cystoplasty. The postoperative urodynamic studies on patients implanted with the engineered bladders showed varying degrees of contractility, capacity, and compliance. The margin between the composite matrix-based engineered segments and the native bladders was grossly indistinguishable during the cystoscopic evaluations. Patients treated with the engineered bladder showed no metabolic consequences or formed urinary calculi. Their levels of mucus production were normal, and renal function was preserved. The biopsies taken from the engineered bladder region showed adequate structural architecture and phenotype. This was the first study in patients demonstrating that engineered bladder tissues can be used in patients requiring bladder reconstruction.

Engineered tissues have also been applied in the urethra. The initial proof of concept studies examining the natural matrix as a biomaterial for urethra reconstruction was conducted on small segment urethral defects in New Zealand white male rabbits (Chen et al. 1999b). Porcine bladder matrices were used to repair a urethral defect in an onlay fashion. Urethrography showed that the reconstructed urethras maintained their diameter over 2 months. Histological evaluation of the retrieved constructs showed vessel formation and a transitional epithelial cell layer by 2 weeks. The smooth muscle layer was formed by 6 months post-implantation.

This basic strategy was used to guide a small clinical study (Atala et al. 1999). Four patients with age ranging from 4 to 20 years with a history of failed surgeries to correct hypospadias underwent reconstructive urethral repair using an acellular collagen matrix derived from cadaveric bladder. The acellular collagen matrix was trimmed to a rectangular shape of the appropriate size ranging 5–15 cm and attached to the urethral plate in an onlay fashion. At 1 year following surgery, retrograde cystourethrography indicated no narrowing of the engineered urethras. Three-year follow-up examinations showed all patients' engineered urethras functioned normally. One patient who received a 15 cm reconstruction developed a subglanular fistula easily repaired by standard methods. Encouraged by the clinical cohort's outcomes, a more extensive study was subsequently conducted (El-Kassaby et al.

2003). A total of 28 patients aged 22–61 with urethral stricture was included in the study. Acellular bladder submucosa was used to repair urethral defects ranging from 1.5 cm to 16 cm in an onlay fashion. Postoperative urethrography showed a patent urethra of wide caliber in all 24 of the successful reconstructions. Average urine flow rates increased from 6 +/− 1.57 ml/sec before the procedure to 9 +/− 1.3 ml/sec postoperatively. Patency of the entire reconstructed sections was confirmed by cystoscopy, and biopsies confirmed the restoration of normal urethral tissue.

While acellular collagen scaffolds were successful for onlay reconstructive procedures in which an intact urethral plate was maintained, studies showed that the use of the material for more complex reconstruction requiring a tubularized urethra resulted in stricture formation. Later studies indicated that, in recellularization of the acellular biomaterial, only implants occurred over short distances from healthy tissue (Dorin et al. 2008). It was found that fibrosis occurred in any biomaterial that was farther than 5 mm from the graft's edge. Onlay reconstruction with acellular matrix was successful because the procedure creates a urethra in which only 10 mm of the circumference of the urethra is comprised of the biomaterial. This allows recellularization to proceed 5 mm from either side of the onlay. Consequently, a study was conducted in rabbits to determine if autologous engineered urethral constructs composed of acellular porcine bladder submucosa seeded with autologous bladder cells could be used to repair complex urethral defects (De Filippo et al. 2002). Porcine bladder matrix was tubularized and seeded with autologous rabbit epithelial cells into the lumen of the scaffolds and smooth muscle cells on the exterior. The seeded grafts of 1 cm in length were used to repair a defect in 12 animals. Normal urethral caliber was maintained in all rabbits that received cell-seeded constructs. Histological analysis of the retrieved constructs demonstrated a normal transitional cell layer surrounded by muscle fiber bundles that became more organized over 6 months following implantation. Organ bath studies performed on retrieved engineered scaffolds showed an appropriate contractile response to chemical and electrical stimuli. This study demonstrated the utility of cell-seeded tubularized engineered urethras for the reconstruction of urethral defects.

A subsequent rabbit study was conducted that demonstrated the feasibility of using cellularized engineered urethral constructs to reconstruct extended urethral defects involving the entire circumference of the urethra (De Filippo et al. 2015). In this study, tubularized acellular bladder submucosa was also used to repair 3 cm long urethral defects in rabbits. All animals that received cellularized engineered constructs had normal urethral caliber across the length of the reconstructed segments. Grossly, the cellularized engineered constructs developed the appearance of normal urethra over time. The lumen of the constructs contained well-organized epithelium, and muscle fiber bundles developed over 6 months following implantation. These results indicate that tubularized collagen-based scaffolds seeded with autologous epithelial and smooth muscle cells can be used to bioengineer a construct to reconstruct a urethral defect much longer than can be accomplished with an acellular biomaterial.

These studies have led to a clinical study in five male pediatric patients with urethral strictures. (Raya-Rivera et al. 2011). Autologous urothelial and smooth

muscle cells were isolated from a 1 × 1 cm bladder biopsy. Cells seeded on tubularized scaffolds were used to repair strictures.

No complications occurred during any of the surgeries. Cystourethroscopy confirmed patent urethras in all patients. Voiding cystourethrograms showed the maintenance of wide caliber urethras in all patients over the period of follow-up. Urethral biopsies showed that the engineered grafts had developed a normal-appearing architecture by 3 months after implantation. This clinical study shows that tubularized urethras can be engineered and remain functional in a clinical setting for up to 6 years, and that the engineered urethras can be used in patients who need complex urethral reconstruction.

12 Conclusions

Recent advances in tissue engineering have provided potential solutions to the existing challenges related to surgical reconstruction. Over the past decades, enormous progress has been made in the area of bladder and urethral reconstruction, experimentally and clinically. This chapter discussed the recent development and the progress of cell-based engineering approaches, covering foundational research to clinical studies. Research related to cells and biomaterials were discussed with a focus on bladder and urethral applications. The development of new and innovative technologies integrating into the tissue building processes has allowed for rapid translation. In addition, support technologies such as bioreactors have further accelerated the development of effective therapies. Continued advancement is expected in all areas leading to the production of viable tissue and organ substitutes through innovation. Although this chapter did not cover 3D bioprinting, it is anticipated that this technology will play a significant role in the tissue engineering field. Studies utilizing the 3D bioprinting technology to build improved lower urinary tract tissues are already being investigated with great interest. The success of tissue engineering will likely depend on innovation.

References

Abbas TO, Yalcin HC, Pennisi CP (2019) From acellular matrices to smart polymers: degradable scaffolds that are transforming the shape of urethral tissue engineering. Int J Mol Sci 20(7):1763

Adamowicz J et al (2019a) Reconstructive urology and tissue engineering: converging developmental paths. J Tissue Eng Regen Med 13(3):522–533

Adamowicz J et al (2019b) Reconstructive urology and tissue engineering: converging developmental paths. J Tissue Eng Regen Med 13(3):522–533

Aguilar MR, San Román J (2019) Chapter 1 - introduction to smart polymers and their applications. In: Aguilar MR, Román JS (eds) Smart polymers and their applications, 2nd edn. Woodhead Publishing, Sawston, pp 1–11

Ahmed S et al (2019) New generation of bioreactors that advance extracellular matrix modelling and tissue engineering. Biotechnol Lett 41(1):1–25

Ajalloueian F et al (2018) Bladder biomechanics and the use of scaffolds for regenerative medicine in the urinary bladder. Nat Rev Urol 15:155

Akkad T et al (2007) Influence of intravesical potassium on pelvic floor activity in women with recurrent urinary tract infections: comparative urodynamics might lead to enhanced detection of dysfunctional voiding. BJU Int 100(5):1071–1074
Alberts B et al (2002) Molecular biology of the cell, vol 91, 4th edn. Garland Science, New York, p 401
Algarrahi K et al (2018) Repair of injured urethras with silk fibroin scaffolds in a rabbit model of onlay urethroplasty. J Surg Res 229:192–199
Anderson CB, McKiernan JM (2018) Surgical complications of urinary diversion. Urol Clin North Am 45(1):79–90
Andersson KE, McCloskey KDJN (2014) Lamina propria: the functional center of the bladder? Neurourol Urodyn 33(1):9–16
Anirudha S et al (2018) Tissue-engineered neo-urinary conduit from decellularized trachea. Tissue Eng Part A 24(19–20):1456–1467
Anumanthan G et al (2008) Directed differentiation of bone marrow derived mesenchymal stem cells into bladder urothelium. J Urol 180(4 Suppl):1778–1783
Atala A (2009) Engineering organs. Curr Opin Biotechnol 20(5):575–592
Atala A (2011) Tissue engineering of human bladder. Br Med Bull 97(1):81–104
Atala A et al (1992) Formation of urothelial structures in vivo from dissociated cells attached to biodegradable polymer scaffolds in vitro. J Urol 148(2 Pt 2):658–662
Atala A et al (1993) Implantation in vivo and retrieval of artificial structures consisting of rabbit and human urothelium and human bladder muscle. J Urol 150(2 Pt 2):608–612
Atala A, Guzman L, Retik AB (1999) A novel inert collagen matrix for hypospadias repair. J Urol 162(3 Pt 2):1148–1151
Atala A et al (2006a) Tissue-engineered autologous bladders for patients needing cystoplasty. Lancet 367(9518):1241–1246
Atala A et al (2006b) Tissue-engineered autologous bladders for patients needing cystoplasty. Lancet 367(9518):1241–1246
Partin AW, Kavoussi LR, Peters CA (2015) Campbell-Walsh urology. In: Atala A (ed) Principles of tissue engineering, vol 1, 11th edn. Elsevier Health Sciences
Bacakova L et al (2019) Versatile application of Nanocellulose: from industry to skin tissue engineering and wound healing. Nanomaterials (Basel) 9(2):164
Badylak SF et al (1989) Small intestinal submucosa as a large diameter vascular graft in the dog. J Surg Res 47(1):74–80
Balsara ZR, Li X (2017) Sleeping beauty: awakening urothelium from its slumber. Am J Physiol Renal Physiol 312(4):F732–F743
Barbagli G et al (2018) Anterior Urethroplasty using a new tissue engineered Oral mucosa graft: surgical techniques and outcomes. J Urol 200(2):448–456
Bharadwaj S et al (2011) Characterization of urine-derived stem cells obtained from upper urinary tract for use in cell-based urological tissue engineering. Tissue Eng Part A
Bharadwaj S et al (2013) Multi-potential differentiation of human urine-derived stem cells: potential for therapeutic applications in urology. Stem Cells
Bodin A et al (2010) Tissue-engineered conduit using urine-derived stem cells seeded bacterial cellulose polymer in urinary reconstruction and diversion. Biomaterials 31(34):8889–8901
Bohne AW, Urwiller KJTJou (1957) Experience with urinary bladder regeneration. J Urol 77(5):725–732
Bouhout S et al (2010) In vitro reconstruction of an autologous, watertight, and resistant Vesical equivalent. Tissue Eng Part A 16(5):1539–1548
Brivanlou AH et al (2003) Stem cells. Setting standards for human embryonic stem cells. Science 300(5621):913–916
Brown RA et al (2005) Ultrarapid engineering of biomimetic materials and tissues: fabrication of Nano- and Microstructures by plastic compression. Adv Funct Mat 15(11):1762–1770
Burdick JA, Prestwich GD (2011) Hyaluronic acid hydrogels for biomedical applications. Adv Mater 23(12):H41–H56
Caione P et al (2012) Bladder augmentation using acellular collagen biomatrix: a pilot experience in exstrophic patients. Pediatr Surg Int 28(4):421–428

Caldamone AA et al (1998) Buccal mucosal grafts for urethral reconstruction. Urology 51(5, Supplement 1):15–19
Caplan AI, Dennis JE (2006) Mesenchymal stem cells as trophic mediators. J Cell Biochem 98(5):1076–1084
Cattan V et al (2011) Mechanical stimuli-induced urothelial differentiation in a human tissue-engineered tubular genitourinary graft. Eur Urol 60(6):1291–1298
Chan BP, Leong KW (2008) Scaffolding in tissue engineering: general approaches and tissue-specific considerations. Eur Spine J 17(Suppl 4):467–479
Chen F, Yoo JJ, Atala AJU (1999a) Acellular collagen matrix as a possible "off the shelf" biomaterial for urethral repair. Urology 54(3):407–410
Chen F, Yoo JJ, Atala A (1999b) Acellular collagen matrix as a possible "off the shelf" biomaterial for urethral repair. Urology 54(3):407–410
Chen C et al (2018) Transplantation of amniotic scaffold-seeded mesenchymal stem cells and/or endothelial progenitor cells from bone marrow to efficiently repair 3-cm circumferential urethral defect in model dogs. Tissue Eng Part A 24(1–2):47–56
Chun SY et al (2007) Identification and characterization of bioactive factors in bladder submucosa matrix. Biomaterials 28(29):4251–4256
Chun SY et al (2015) Urethroplasty using autologous urethral tissue-embedded acellular porcine bladder submucosa matrix grafts for the management of long-segment urethral stricture in a rabbit model. J Korean Med Sci 30(3):301–307
Chunxiang F et al (2019) Electrospun nanofibers with core–shell structure for treatment of bladder regeneration. Tissue Eng Part A 25:1289–1299
Cilento BG et al (1994) Phenotypic and cytogenetic characterization of human bladder urothelia expanded in vitro. J Urol 152(2 Pt 2):665–670
Coutu DL et al (2014) Tissue engineering of rat bladder using marrow-derived mesenchymal stem cells and bladder acellular matrix. PLoS One 9(12):e111966
Crapo PM, Gilbert TW, Badylak SFJB (2011) An overview of tissue and whole organ decellularization processes. Biomaterials 32(12):3233–3243
Dahl SLM et al (2011a) Readily available tissue-engineered vascular grafts. Sci Transl Med 3(68):68ra9-68ra9
Dahl SL et al (2011b) Readily available tissue-engineered vascular grafts. Sci Transl Med 3(68):68ra9-68ra9
Davis NF et al (2018a) Tissue engineered extracellular matrices (ECMs) in urology: evolution and future directions. Surgeon 16(1):55–65
Davis NF et al (2018b) The role of stem cells for reconstructing the lower urinary tracts. Curr Stem Cell Res Ther 13(6):458–465
de Bournonville S et al (2018) 233 - a modular, standalone perfusion bioreactor for robust, monitored and controlled tissue engineering. Cytotherapy 20(5 Supplement):S81–S82
De Coppi P et al (2007a) Isolation of amniotic stem cell lines with potential for therapy. Nat Biotechnol 25(1):100–106
De Coppi P et al (2007b) Amniotic fluid and bone marrow derived mesenchymal stem cells can be converted to smooth muscle cells in the cryo-injured rat bladder and prevent compensatory hypertrophy of surviving smooth muscle cells. J Urol 177(1):369–376
De Filippo RE, Yoo JJ, Atala A (2002) Urethral replacement using cell seeded tubularized collagen matrices. J Urol 168(4 Pt 2):1789–1792; discussion 1792-3
De Filippo RE et al (2015) Penile urethra replacement with autologous cell-seeded tubularized collagen matrices. J Tissue Eng Regen Med 9(3):257–264
Deng K et al (2014) Mesenchymal stem cells and their secretome partially restore nerve and urethral function in a dual muscle and nerve injury stress urinary incontinence model. Am J Physiol Renal Physiol 308(2):F92–F100
Dhandayuthapani B et al (2011a, 2011) Polymeric scaffolds in tissue engineering application: a review. Int J Polym Sci
Dhandayuthapani B et al (2011b) Polymeric scaffolds in tissue engineering application: a review. Int J Polym Sci

Dorin RP et al (2008) Tubularized urethral replacement with unseeded matrices: what is the maximum distance for normal tissue regeneration? World J Urol 26(4):323–326
El-Kassaby AW et al (2003) Urethral stricture repair with an off-the-shelf collagen matrix. J Urol 169(1):170–173; discussion 173
El-Taji OM, Khattak AQ, Hussain SA (2015) Bladder reconstruction: the past, present and future. Oncol Lett 10(1):3–10
Farhat WA, Yeger HJWJoU (2008) Does mechanical stimulation have any role in urinary bladder tissue engineering? World J Urol 26(4):301–305
Feil G et al (2006) Investigations of Urothelial cells seeded on commercially available small intestine submucosa. Eur Urol 50(6):1330–1337
Feng C et al (2011) Reconstruction of three-dimensional neourethra using lingual keratinocytes and corporal smooth muscle cells seeded acellular corporal spongiosum. Tissue Eng Part A
Fiala R et al (2007) Porcine small intestinal submucosa graft for repair of anterior urethral strictures. Eur Urol 51(6):1702–1708
Filippo RED, Yoo JJ, Atala A (2002) Urethral replacement using cell seeded tubularized collagen matrices. J Urol 168(4 Part 2):1789–1793
Fu Q et al (2007) Urethral replacement using epidermal cell-seeded tubular acellular bladder collagen matrix. BJU Int 99(5):1162–1165
Fu W-J et al (2011) A surface-modified biodegradable urethral scaffold seeded with urethral epithelial cells. Chin Med J 124(19):3087–3092
Furthmayr H, Timpl R (1976) Immunochemistry of collagens and procollagens. In: Hall DA, Jackson DS (eds) International review of connective tissue research. Elsevier, pp 61–99
Gabr H et al (2018) 234 - tissue engineering for bladder reconstruction: exploring bioscaffolds. Cytotherapy 20(5, Supplement):S82
Gabrich PN et al (2007) Avaliação das medidas do comprimento peniano de crianças e adolescentes. J Pediatr 83:441–446
Griffith LG, Naughton G (2002) Tissue engineering–current challenges and expanding opportunities. Science 295(5557):1009–1014
Gu GL et al (2012) Tubularized urethral replacement using tissue-engineered peritoneum-like tissue in a rabbit model. Urol Int 89(3):358–364
Haberstroh KM et al (2002) A novel in-vitro system for the simultaneous exposure of bladder smooth muscle cells to mechanical strain and sustained hydrostatic pressure. J Biomech Eng 124(2):208–213
Hardy JG et al (2015) Electroactive tissue scaffolds with aligned pores as instructive platforms for biomimetic tissue engineering. Bioengineering 2(1):15–34
Hayn MH, Bellinger MF, Schneck FX (2009) Small intestine submucosa as a corporal body graft in the repair of severe Chordee. Urology 73(2):277–279
Horst M et al (2013a) Engineering functional bladder tissues. J Tissue Eng Regen Med 7(7):515–522
Horst M et al (2013b) Engineering functional bladder tissues. J Tissue Eng Regen Med 7(7):515–522
Horst M et al (2013c) A bilayered hybrid microfibrous PLGA–acellular matrix scaffold for hollow organ tissue engineering. Biomaterials 34(5):1537–1545
Horst M et al (2019) Tissue engineering in pediatric bladder reconstruction – the road to success. Front Pediatr 7:91
Huang J-W et al (2015) Urethral reconstruction with a 3D porous bacterial cellulose scaffold seeded with lingual keratinocytes in a rabbit model. Biomed Mater 10(5):055005
Hubbell JA (2003) Materials as morphogenetic guides in tissue engineering. Curr Opin Biotechnol 14(5):551–558
Husmann DA, Rathbun SR (2006) Long-term followup of visual internal urethrotomy for management of short (less than 1 cm) penile urethral strictures following hypospadias repair. J Urol 176(4, Supplement):1738–1741
Jia W et al (2015) Urethral tissue regeneration using collagen scaffold modified with collagen binding VEGF in a beagle model. Biomaterials 69:45–55

Kajbafzadeh A-M et al (2011) In-vivo autologous bladder muscular wall regeneration: application of tissue-engineered pericardium in a model of bladder as a bioreactor. J Pediatr Urol 7(3):317–323
Kajbafzadeh A-M et al (2017) Future prospects for human tissue engineered urethra transplantation: decellularization and recellularization-based urethra regeneration. Reprod Tissue Eng 45(7):1795–1806
Keays MA, Dave S (2017) Current hypospadias management: diagnosis, surgical management, and long-term patient-centred outcomes. Can Urol Assoc J 11(1-2Suppl1):S48–S53
Kim Y-J, Matsunaga YT (2017) Thermo-responsive polymers and their application as smart biomaterials. J Mater Chem B 5(23):4307–4321
Kingham PJ et al (2014) Stimulating the neurotrophic and angiogenic properties of human adipose-derived stem cells enhances nerve repair. Stem Cells Dev 23(7):741–754
Kirkton RD et al (2019) Bioengineered human acellular vessels recellularize and evolve into living blood vessels after human implantation. Sci Transl Med 11(485):eaau6934
Koie T et al (2018) Robotic cross-folded U-configuration intracorporeal ileal neobladder for muscle-invasive bladder cancer: initial experience and functional outcomes. Int J Med Robot Comp Assisted Surg 14(6):e1955
Kowalski PS et al (2018) Smart biomaterials: recent advances and future directions. ACS Biomater Sci Eng 4(11):3809–3817
Kropp BP et al (1996) Regenerative urinary bladder augmentation using small intestinal submucosa: urodynamic and histopathologic assessment in long-term canine bladder augmentations. Investig Urol 155(6):2098–2104
Lazzeri M et al (2016) Incidence, causes, and complications of urethral stricture disease. Eur Urol Suppl 15(1):2–6
Lee KY, Mooney DJ (2012) Alginate: properties and biomedical applications. Prog Polym Sci 37(1):106–126
Li C et al (2008) Urethral reconstruction using oral keratinocyte seeded bladder acellular matrix grafts. J Urol 180(4):1538–1542
Liu JS et al (2016) Bone marrow stem/progenitor cells attenuate the inflammatory milieu following substitution Urethroplasty. Sci Rep 6:35638
Lu S-H et al (2005) Biaxial mechanical properties of muscle-derived cell seeded small intestinal submucosa for bladder wall reconstitution. Biomaterials 26(4):443–449
Lv X et al (2016) Electrospun poly(l-lactide)/poly(ethylene glycol) scaffolds seeded with human amniotic mesenchymal stem cells for urethral epithelium repair. Int J Mol Sci 17(8):1262
Lv X et al (2018) A smart bilayered scaffold supporting keratinocytes and muscle cells in micro/nano-scale for urethral reconstruction. Theranostics 8(11):3153–3163
Magnan M et al (2009) Tissue engineering of a genitourinary tubular tissue graft resistant to suturing and high internal pressures. Tissue Eng Part A 15(1):197–202
Mangir N et al (2019a) Current state of urethral tissue engineering. Curr Opin Urol 29(4):385–393
Mangır N et al (2016) Production of ascorbic acid releasing biomaterials for pelvic floor repair. Acta Biomater 29:188–197
Mangır N et al (2017) Oestradiol-releasing biodegradable mesh stimulates collagen production and angiogenesis: an approach to improving biomaterial integration in pelvic floor repair. Eur Urol Focus 5(2):280–289
Mangir N et al (2019b) Current state of urethral tissue engineering. Curr Opin Urol 29(4):385–393
Markiewicz MR et al (2008) Morbidity associated with oral mucosa harvest for urological reconstruction: an overview. J Oral Maxillofac Surg 66(4):739–744
Martin I, Wendt D, Heberer M (2004) The role of bioreactors in tissue engineering. Trends Biotechnol 22(2):80–86
Mijailovich SM et al (2007) Effect of urethral compliance on the steady state p-Q relationships assessed with a mechanical analog of the male lower urinary tract. Neurourol Urodyn 26(2):234–246
Nagele U et al (2008) In vitro investigations of tissue-engineered multilayered Urothelium established from bladder washings. Eur Urol 54(6):1414–1422

Neuhof H (1917) Facial transplantation into visceral defects: an experimental and clinical study. Surg Gynecol Obstet 25:383–427
Niklason L et al (1999) Functional arteries grown in vitro. Science 284(5413):489–493
Oberpenning F et al (1999a) De novo reconstitution of a functional mammalian urinary bladder by tissue engineering. Nat Biotechnol 17(2):149
Oberpenning F et al (1999b) De novo reconstitution of a functional mammalian urinary bladder by tissue engineering. Nat Biotechnol 17(2):149–155
Ohel G, Haddad S, Samueloff A (1995) Fetal urine production and micturition and fetal behavioral state. Am J Perinatol 12(2):91–92
Orabi H et al (2013a, 2013) Tissue engineering of urinary bladder and urethra: advances from bench to patients. Sci World J:154564–154564
Orabi H et al (2013b) The use of small intestinal submucosa graft for hypospadias repair: pilot study. Arab J Urol 11(4):415–420
Osborn SL et al (2014) Induction of human embryonic and induced pluripotent stem cells into urothelium. Stem Cells Transl Med 3(5):610–619
Ouellet G et al (2011) Production of an optimized tissue-engineered pig connective tissue for the reconstruction of the urinary tract. Tissue Eng Part A 17(11–12):1625–1633
Palminteri E et al (2012) Long-term results of small intestinal submucosa graft in bulbar urethral reconstruction. Urology 79(3):695–701
Partin, A.W., et al., Campbell-Walsh urology, 11th Edition. Principles of tissue engineering, ed. A. Atala. Vol. 1. 2015: Elsevier Health Sciences. 482–498
Pi Q et al (2018) Digitally tunable microfluidic bioprinting of multilayered Cannular tissues. Adv Mater 30(43):e1706913
Pinnagoda K et al (2016) Engineered acellular collagen scaffold for endogenous cell guidance, a novel approach in urethral regeneration. Acta Biomater 43:208–217
Poole Wilson DS, Barnard RJBjou (1971) Total cystectomy for bladder tumors. Br J Urol 43(1):16–23
Probst M et al (1997) Reproduction of functional smooth muscle tissue and partial bladder replacement. Br J Urol 79(4):505–515
Rajasekaran M, Stein P, Parsons CLJIjou (2006) Toxic factors in human urine that injure urothelium. Int J Urol 13(4):409–414
Ramuta TŽ, Kreft ME (2018) Human amniotic membrane and amniotic membrane–derived cells: how far are we from their use in regenerative and reconstructive urology? Cell Transplant 27(1):77–92
Rashidbenam Z et al (2019) Overview of urethral reconstruction by tissue engineering: current strategies, clinical status and future direction. Tissue Eng Regen Med
Raya-Rivera A et al (2011) Tissue-engineered autologous urethras for patients who need reconstruction: an observational study. Lancet 377(9772):1175–1182
Roelofs LAJ, Oosterwijk E et al (2016) Bladder regeneration using a smart Acellular collagen scaffold with growth factors VEGF, FGF2 and HB-EGF. Tissue Eng Part A 22(1–2):83–92
Roth CC, Kropp BPJCUR (2009) Recent advances in urologic tissue engineering. Curr Urol Rep 10(2):119–125
Rousseau A et al (2015) Adipose-derived stromal cells for the reconstruction of a human vesical equivalent. J Tissue Eng Regen Med 9(11):E135–E143
Rouwkema J, Rivron NC, van Blitterswijk CA (2008) Vascularization in tissue engineering. Trends Biotechnol 26(8):434–441
Saif A et al (2019) Augmentation cystoplasty of diseased porcine bladders with bi-layer silk fibroin grafts. Tissue Eng Part A 25(11–12):855–866
Salem SA et al (2013) Human adipose tissue derived stem cells as a source of smooth muscle cells in the regeneration of muscular layer of urinary bladder wall. Malay J Med Sci MJMS 20(4):80–87
Sanchez RP et al (1958) Vesical regeneration in the human after total cystectomy and implantation of a plastic mould. Br J Urol 30(2):180–188

Schaefer M et al (2013) Bladder augmentation with small intestinal submucosa leads to unsatisfactory long-term results. J Pediatr Urol 9(6, Part A):878–883
Serrano-Aroca Á, Vera-Donoso CD, Moreno-Manzano V (2018a) Bioengineering approaches for bladder regeneration. Int J Mol Sci 19(6):1796
Serrano-Aroca A, Vera-Donoso CD, Moreno-Manzano V (2018b) Bioengineering approaches for bladder regeneration. Int J Mol Sci 19(6)
Shaikh FM et al (2010) New pulsatile hydrostatic pressure bioreactor for vascular tissue-engineered constructs. Artif Organs 34(2):153–158
Shakhssalim N et al (2013) Bladder smooth muscle cells interaction and proliferation on PCL/PLLA electrospun Nanofibrous scaffold. Int J Artif Organs 36(2):113–120
Shiroyanagi Y et al (2003) Transplantable Urothelial cell sheets harvested noninvasively from temperature-responsive culture surfaces by reducing temperature. Tissue Eng 9(5):1005–1012
Shirozu H et al (1995) Penile tumescence in the human fetus at term — a preliminary report. Early Hum Dev 41(3):159–166
Shukla D et al (2008a) Bone marrow stem cells for urologic tissue engineering. World J Urol 26(4):341–349
Shukla D et al (2008b) Bone marrow stem cells for urologic tissue engineering. World J Urol 26(4):341
Sivaraman S et al (2019) Evaluation of poly (carbonate-urethane) urea (PCUU) scaffolds for urinary bladder tissue engineering. Ann Biomed Eng 47(3):891–901
Stangel-Wojcikiewicz K et al (2014) Autologous muscle-derived cells for the treatment of female stress urinary incontinence: a 2-year follow-up of a polish investigation. Neurourol Urodyn 33(3):324–330
Stock UA, Vacanti JP (2001) Cardiovascular physiology during fetal development and implications for tissue engineering. Tissue Eng 7(1):1–7
StÖlting MNL et al (2016) Magnetic stimulation supports muscle and nerve regeneration after trauma in mice. Muscle Nerve 53(4):598–607
Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126(4):663–676
Taylor DA et al (2018) Decellularized matrices in regenerative medicine. Acta Biomater 74:74–89
Theocharis AD et al (2016) Extracellular matrix structure. Adv Drug Deliv Rev 97:4–27
Wang Y et al (2008) In vivo degradation of three-dimensional silk fibroin scaffolds. Biomaterials 29(24):3415–3428
Wang Y et al (2019) Bioengineered bladder patches constructed from multilayered adipose-derived stem cell sheets for bladder regeneration. Acta Biomater 85:131–141
Wu S et al (2011) Human urine-derived stem cells seeded in a modified 3D porous small intestinal submucosa scaffold for urethral tissue engineering. Biomaterials 32(5):1317–1326
Wu Y et al (2016) Electroactive biodegradable polyurethane significantly enhanced Schwann cells myelin gene expression and neurotrophin secretion for peripheral nerve tissue engineering. Biomaterials 87:18–31
Xie M et al (2013) Evaluation of stretched electrospun silk fibroin matrices seeded with urothelial cells for urethra reconstruction. J Surg Res 184(2):774–781
Yalla SV, Burros HM (1974) Conduit and regional compliance of female canine urethra: observations during voiding and passive urethral infusion. Urology 4(2):155–161
Yao C et al (2013) Nanostructured polyurethane-poly-lactic-co-glycolic acid scaffolds increase bladder tissue regeneration: an in vivo study. Int J Nanomedicine 8:3285–3296
Yoo JJ et al (1998) Bladder augmentation using allogenic bladder submucosa seeded with cells. Urology 51(2):221–225
Zhang YY, Frey P (2003) Growth of cultured human urothelial cells into stratified urothelial sheet suitable for autografts. Adv Exp Med Biol 539(Pt B):907–920
Zhang Y et al (2000) Coculture of bladder urothelial and smooth muscle cells on small intestinal submucosa: potential applications for tissue engineering technology. J Urol 164(3 Part 2): 928–935

Zhang Y et al (2005) Growth of bone marrow stromal cells on small intestinal submucosa: an alternative cell source for tissue engineered bladder. BJU Int 96(7):1120–1125
Zhang Y et al (2006a) Challenges in a larger bladder replacement with cell-seeded and unseeded small intestinal submucosa grafts in a subtotal cystectomy model. BJU Int 98(5):1100–1105
Zhang Y et al (2006b) Challenges in a larger bladder replacement with cell-seeded and unseeded small intestinal submucosa grafts in a subtotal cystectomy model. BJU Int 98(5):1100–1105
Zhang Y et al (2008) Urine derived cells are a potential source for urological tissue reconstruction. J Urol 180(5):2226–2233
Zhang M et al (2013) Differentiation of human adipose-derived stem cells co-cultured with Urothelium cell line toward a Urothelium-like phenotype in a nude murine model. Urology 81(2):465.e15–465.e22
Zhang L et al (2016) Development of a cell-seeded modified small intestinal submucosa for urethroplasty. Heliyon 2(3):e00087
Zhe W et al (2016) Smooth muscle precursor cells derived from human pluripotent stem cells for treatment of stress urinary incontinence. Stem Cells Dev 25(6):453–461
Zhou S et al (2017) Fabrication of tissue-engineered bionic urethra using cell sheet technology and labeling by ultrasmall Superparamagnetic Iron oxide for full-thickness urethral reconstruction. Theranostics 7(9):2509–2523