Bladder

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8.1 Introduction

The bladder is susceptible to a variety of congenital anomalies, injuries, and disorders, such as cancer, trauma, infection, and chronic inflammation [1]. In cases of muscle-invasive and refractory non-muscle-invasive bladder cancer and end-stage (congenital) bladder disease, the current standard treatment is radical cystectomy in combination with urinary diversion or bladder augmentation, which serves to restore bladder capacity and compliance and prevent vesicoureteral reflux and renal damage [2, 3]. The method of diversion depends in part on the nature of defect and the patient's needs and wishes. Bladder reconstruction is one of the greatest surgical challenges in the field of urology. To repair or replace the bladder, gastrointestinal segments are commonly used. However, gastrointestinal tissues are designed to absorb specific solutes, whereas bladder tissues are designed to excrete solutes. Conventional bladder reconstruction using gastrointestinal tissue (enterocystoplasty) is associated with numerous complications, including mucus production, bacterial colonization, electrolyte imbalance, anastomotic leakage, enteric fistulae, bowel obstruction, and prolonged episodes of ileus, intestinal failure, or malignancy, as well as significant morbidity and functional alterations [4]. To overcome these problems, numerous alternative reconstructive procedures have been introduced including autoaugmentation and ureterocystoplasty [5–7], as well as tissue expansion [8, 9]. However, almost all of them resulted in failure due to technical difficulty or complication. Tissue engineering techniques, transplantation of biomaterials seeded with cells, have been developed as a promising alternative that circumvent many of the limitations and complications associated with enterocystoplasty; therefore, it is considered a potentially promising option for bladder reconstruction.

Regenerative medicine aims to regenerate tissues and organs by creating biological equivalents through supplementation of scaffolding materials with bioactive components, cells, or a combination thereof [10]. Various attempts have been made to reconstruct the bladder in both animal and human studies using regenerative medicine techniques [11]. In 2006, the first clinical application of regenerative medicine for bladder reconstruction was published by Atala et al. [12] In this study, they implanted a collagen/polyglycolic acid composite scaffold with urothelial and smooth muscle cells (SMCs) into the bladder dome area after partial cystectomy.

The fields of tissue engineering and regenerative medicine have achieved substantial progression over the previous two decades. It

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encompasses the cell biology, transplantation, material science, and biomedical engineering, toward identifying alternatives that can reestablish and preserve the regular function of damaged tissues and organs [13]. Studies for bladder tissue engineering strategies have been orientated in two directions: firstly, to identify the most appropriate type of cells for regeneration and to proficiently incorporate it into bladder cells and, secondly, to determine the most appropriate biomaterial and technique of embedding cells into engineered grafts [14, 15]. The selected grafts must exhibit all the qualities of the native tissue, acting ultimately as microenvironments for the implanted cells to proliferate and differentiate. Numerous studies have been tried to identify ideal materials for bladder regeneration including acellular tissue matrices and synthetic matrices [16]. For example, bladder submucosa matrix (BSM), obtained by decellularization of bladder submucosa, has been shown to be an effective material for clinical applications because it maintains the extracellular matrix of the bladder as well as the biological activity of growth factors [17].

On the other hand, efforts have been exerted to find ideal cell source for bladder regeneration. Autologous bladder cells are the gold standard source for bladder regeneration in cell-based tissue engineering. To provide functional cells for tissue reconstruction and remodeling, cultured cells at early passage (before passage 5) provide optimal results. In addition, cells should be cultured on the scaffold for a short time (less than 2 weeks) since SMCs lose their phenotype after long-term static culture [18]. Mechanical preconditioning of muscle cells might be an alternative culture method that would maintain the phenotype and functional characteristics of cells before implantation [19]. Immortalized muscle cell lines are attractive since they may generate a large number of functional cells. However, these types of cell lines have limited clinical application, as immortalized cells carry the risk of tumor formation [20]. For patients with bladder cancer, normal bladder cells are not available; thus, an alternative cell source needs to be found, and several cell types are being investigated for this purpose, including bone marrow stem cells, skeletal myoblasts, and adipose progenitor cells [21–24].

In the 1950s, efforts to find alternatives to intestinal cystoplasty included the use of alloplastic materials [25–31]. Although these early research efforts met with very limited success owing to foreign body complications, they provided the foundation for regenerative medicine and led to a focus on biodegradable, collagen-rich tissues for use as a scaffold with and without cell seeding [32–38]. Atala et al. [12] reported the first data regarding the use of a seeded biodegradable construct for augmentation of the neurogenic cell population. This work led to an industry-supported, prospective, multi-institution investigation using a neo-bladder construct. Unfortunately, compliance improved in only four patients after 12 months and in five after 36 months but in none to a clinically or statistically significant degree. Capacity also did not improve [39]. Using autologous tissue from a neuropathic source may lower the yield of usable cells or could potentiate the growth of abnormal tissue [40]. An alternate cell source for regenerative tissue, such as stem cells, might circumvent these concerns. However, the use of stem cells carries its own set of obstacles [41–44]. Further investigation into the clinical applicability of regenerative tissue engineering is required, but this technology remains poised to alter reconstructive bladder surgery.

This chapter is focused on current status of regenerative medicine including achievements and perspectives on the use of biomaterials and stem cells in the field of bladder reconstruction.

8.2 The Use of Matrices for Bladder Regeneration

The bladder is considered as a good candidate for regenerative medicine-based approach because it is one of the simple structured organs (not so many different kinds of cells, minimal metabolic activity, and less interaction with adjacent organs). Therefore, many researchers have studied bladder reconstruction with various methods.

One of the approaches is implantation of biomaterials into the bladder. In this approach, selection of the appropriate bioscaffold for large bladder tissue replacement is a critical component. There are distinct benefits to using biocompatible material in regenerative medicine for the purpose of cell delivery vehicles and for bearing the physical maintenance required for tissue replacement [45]. Scaffolds are constructs that are designed to direct tissue development and the growth of cells during the process of healing [46]. Bladder replacements should therefore provide provisional mechanical support, adequate to endure forces exerted from neighboring structures, while maintaining a potential zone for tissue development. Biomaterials used for bladder replacements should possess the ability to be easily manipulated into a hollow, spherical configuration. Furthermore, the biomaterials should possess the ability to biodegrade for complete tissue development, without causing inflammation. An ideal scaffold for bladder tissue reconstruction should have an adjustable 3-D porous structure, high mechanical strength, uniformity, and appropriate degradation rate, and it should be easily fabricated. Over the last few decades, several bladder wall substitutes, composed of both synthetic and organic materials, have been investigated extensively. Biocompatible synthetic materials are considered potential substitutes for bladder tissue. Biomaterials can be divided into four main categories: (1) naturally derived matrices, including collagen; (2) synthetic matrices, including polylactic-co-glycolic acid (PLGA); (3) acellular tissue matrices, including bladder submucosa; and (4) hybrid or composite scaffold [47].

8.2.1 Naturally Derived Matrices

Collagen, a naturally derived matrix, is considered to be the most ubiquitous protein in the human body, and it is often used alongside alginate as a natural matrix. It is useful in tissue engineering, as it possesses the ability to be easily manipulated and does not provoke an immune response [48]. Through the use of innovative inkjet technology, it has been possible to use bioprinting to create a naturally derived 3-D construct, with a precise arrangement of growth factors and other cellular components, into a patient-specific scaffold [16].

8.2.2 Synthetic Matrices

Synthetic polymers are promising materials because of their reproducibility, large-scale production, mechanical properties, ease of fabrication, and manipulable strength, degradation rate, and microstructure [49]. The typical synthetic biomaterials utilized for urinary tissue regeneration are polyglycolic acid (PGA) and polylactic-co-glycolic acid (PLGA), which are biocompatible, biodegradable, and FDA-approved for human applications [1]. However, the brittleness of these synthetic polymers and the acidic by-products produced during their degradation have been linked to inflammation and immune dysfunction [50–54]. Furthermore, studies have shown that stiff synthetic materials can cause mechanical failure and urinary stone formation, and over time, the degradation products cause fibroblast deposition, scarring, and graft contracture and reduce reservoir volume [25, 55, 56]. To avoid these side effects, interest in polycaprolactone (PCL) has increased because it is flexible, biocompatible, stable, and resistant to resorption; its degradation products show low toxicity; and it could help protect the urothelium [57, 58]. However, it is well known that the use of scaffolds with low hydrophilicity (hydrophobic), like PCL, can lead to low initial cell seeding efficacy and heterogeneous/slow cell growth due to inadequate diffusion of the cell culture medium into the scaffold and the lack of specific interaction sites with cells [59]. Besides abovementioned biomaterials, numerous synthetic materials including polyvinyl sponge, Teflon, collagen matrices, Vicryl PGA matrices, and silicone have been studied in both experimental and clinical settings. However, most of these materials failed to show possibilities of clinical application due to mechanical, structural, functional, or biocompatibility problems. Permanent synthetic materials

often succumb to mechanical failure and urinary stone formation, while degradable materials lead to fibroblast deposition, scarring, graft contracture, and a reduced reservoir volume [60, 61].

8.2.3 Acellular Tissue Matrices

Decellularized matrices are the most commonly used naturally derived urological matrices. They are usually harvested from autologous, allogenic, or xenogeneic tissue [55, 62]. Chemical or mechanical processing decellularizes the matrix, removing all cellular components and leaving a natural platform for tissue development [63]. The most common origin of decellularized matrices is the tissue harvested from the bladder or small intestinal mucosa.

Bladder submucosa matrix (BSM), obtained by decellularization of bladder submucosa, has been shown to be an effective material for clinical applications because it maintains the extracellular matrix of the bladder as well as the biological activity of growth factors [17]. Non-seeded allogeneic acellular bladder matrices have been used as scaffolds for the ingrowth of host bladder wall components. These matrices are prepared by mechanically and chemically removing all cellular components from bladder tissue. Probst et al. reported a method of bladder augmentation that avoided the complications encountered with the use of bowel segments using a newly developed acellular biomaterial, the bladder acellular matrix graft (BAMG), as a homologous graft [64]. Thirty-four rats underwent a partial cystectomy (40–50%) and grafting with a BAMG of equal size. After initial bladder enlargement, the graft was progressively infiltrated by host vessels and SMCs, and the mucosal lining was complete within 10 days. After 4 weeks, histological analysis showed that all bladder wall components were present in the graft. Ingrowth was complete after 8 weeks, except for neural regeneration, which was only partial. At 12 weeks, the bladder wall muscle structure in the graft was so well developed that it was difficult to delineate the junction between the host bladder and BAMG. Neural regeneration continued to improve, and normal bladder capacity was maintained throughout the study. They concluded that the BAMG appeared to serve, without rejection, as a collagen and elastin framework for the ingrowth of all bladder wall components.

Small intestinal submucosa (SIS), a biodegradable, acellular, xenogeneic collagen-based tissue matrix graft, was first described in the early 1960s as an acellular matrix for tissue replacement in the vascular field [65]. The matrix is derived from pig small intestine in which the mucosa is mechanically removed from the inner surface and the serosa and muscular layer are removed from the outer surface. Animal studies have shown that, when used for bladder augmentation, non-seeded SIS matrix is able to regenerate [66]. In this study, 15-month small intestinal submucosa-regenerated canine bladder strips in vitro were analyzed by muscle bath compliance, contractility testing, and immunohistochemical staining, and the results showed that small intestinal submucosa-regenerated canine bladder. Compliance studies demonstrated no significant difference between small intestinal submucosa-regenerated and control bladders, which were 30-fold more compliant than a native small intestinal submucosal graft. Contractility studies demonstrated that the contractile responses and innervation were similar to those of normal canine bladder. Afferent nerves were observed by immunohistochemical techniques. Histologically, the transitional layer was the same as that of the native bladder tissue; however, as with other non-seeded collagen matrices, the muscle layer was not fully developed. In vitro contractility studies performed on SIS-regenerated dog bladders showed a 50% decrease in maximal contractile response compared to that of normal bladder tissues.

Bladder augmentation with porcine acellular bowel tissue matrix, human placental membranes, or porcine SIS was performed using laparoscopic techniques in minipigs. At 12 weeks

post-op, the grafts had contracted to 60% of their original size, and histologically, the grafts showed predominantly mucosal regeneration [67]. The human placental membranes, acellular tissue matrices, and SIS grafts persisted as well-vascularized fibrous bands, without evidence of significant inflammatory responses. These results suggest that a laparoscopic technique for partial bladder wall replacement using a cell-free graft is feasible. At 1 year, the hemicystectomy and bladder replacement with SIS group showed muscle tissue at the graft periphery and center; however, it consisted of small fused bundles with significant fibrosis. Compared with primary bladder closure after hemicystectomy, there was no improvement in bladder capacity or compliance [68]. In this study, 12 minipigs underwent laparoscopic hemicystectomy. Then, six pigs underwent bladder reconstruction with SIS and ipsilateral ureteral reimplantation. In the SIS group, four out of five surviving pigs had unobstructed reimplanted ureters without evidence of hydroureteronephrosis, while one had a high-grade obstruction at the reimplantation site. Histopathologic studies 1 year after implantation revealed muscle at the graft periphery and center; however, it consisted of small fused bundles with significant fibrosis. Although nerves were present at the graft periphery and center, they were decreased in number. Compared to primary bladder closure, no increase in bladder capacity or compliance was observed.

Studies of acellular matrices that may provide the necessary environment to promote cell migration, growth, and differentiation have been conducted. Chin et al. identified and characterized the bioactive factors in decellularized BSM by using ELISA, Western blotting, and immunohistochemistry for its effective utilization in regenerative medicine [17]. At least ten growth factors, including VEGF, BMP4, PDGF-BB, KGF, TGF-beta 1, IGF, bFGF, EGF, and TGF-alpha, were detected in the decellularized BSM. The presence of collagen (types 1, 2, 3, 4), laminin, and elastin within the matrix was also demonstrated. Supplementation with soluble BSM extracts showed an obvious effect on cell proliferation. These findings demonstrate that the growth factors and extracellular matrix components in the BSM maintain biological activity even after decellularization and extraction, supporting the wide applicability of BSM in tissue regeneration. The identification and characterization of the growth factors and extracellular matrix components in BSM are a prerequisite for understanding tissue regeneration using this scaffold. A study by Kikuno et al. showed that grafts of acellular collagen matrices can be enhanced by adding growth factors to improve bladder regeneration [69]. They evaluated the combined effects of nerve growth factor (NGF) and vascular endothelial growth factor (VEGF) on regeneration of the BAMG in spinal cord injury (SCI)-mediated neurogenic bladder in rats. Bladder capacity and compliance were significantly increased in all BAMG groups 8 weeks after surgery compared with that before bladder replacement surgery. However, bladder capacity and compliance were much higher in the VEGF and NGF group than in the control, NGF alone, and VEGF alone groups. NGF had a significant synergistic effect on the development, differentiation, and functional restoration of the BAMG when administered with VEGF in neurogenic bladders. Therefore, NGF may be a useful cytokine for enhancing the regeneration of a functional bladder following acellular matrix grafting in a neurogenic rat model.

8.2.4 Hybrid or Composite Scaffolds

While it is difficult to find a single material that satisfies the numerous requirements for bladder regeneration, composite materials might have the necessary characteristics for use as scaffolds [70]. Tensile strength and elastic modulus are important properties for urine storage and expulsion, and changes in the viscoelastic properties of the bladder wall can lead to voiding dysfunction [71]. Therefore, composite scaffolds which have many benefits, including reproducibility, large-scale production, and

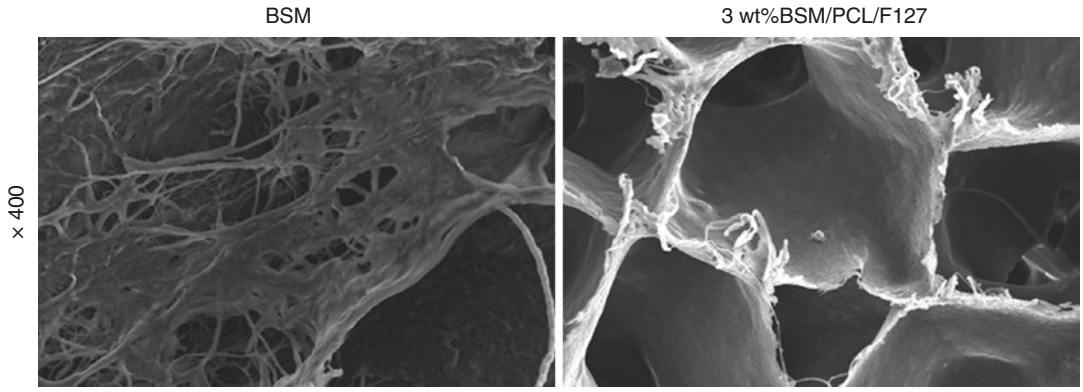

Fig. 8.1 Field emission scanning electron microscopy images of cell morphology on a 3 wt% BSM composite scaffold. For the analyses, upper urinary tract-derived urine stem cells were used. The BSM scaffold was used as the control. *PCL* polycaprolactone, *F127* Pluronic F127 *BSM* bladder submucosa matrix

appropriate mechanical properties, as well as adjustable strength, degradation rate, and microstructure, may be useful synthetic polymers, because the desirable configuration is easily achieved. Pluronic F127 (F127) does not alter the properties of the scaffold, but it improves hydrophilicity and the microenvironment for cell attachment [72]. PCL was investigated as a potential substitute for bladder tissue reconstruction because of its flexibility, biocompatibility, stability, and resistance to resorption [57]. Jang et al. [1] fabricated a blend of PCL with F127 and BSM, which has been shown to be a nonimmunogenic, noncytotoxic, collagen-rich membrane that can be rapidly replaced by native tissues. In their study, the artificial polymer PCL/F127 was chosen for its natural elastic characteristics and physiochemical properties, which enable fabrication of a reservoir of sufficient volume, and naturally occurring BSM was used to improve its biocompatibility by increasing the proliferation of the urothelium and SMCs [1]. Testing for the proper PCL/F127/BSM ratio for the scaffold showed that a PCL/F127/3 wt% BSM composite scaffold exhibited significantly enhanced hydrophilicity, the surface was easily immobilized, and there was no evidence of teratoma formation in vivo. The cell morphology on the 3 wt% BSM composite scaffold was assessed by field emission scanning electron microscopy (FE-SEM; Fig. 8.1). The BSM scaffold was used as the control. The primary upper urinary tract-derived urine stem cells (uUSCs) on the 3 wt% BSM/F127 composite scaffold were flattened, with cell processes that extended to the surface of the scaffold. A similar morphology was seen in the control. FE-SEM analysis showed that the uUSCs were tightly attached to the surface of the scaffold and had extended into the pores on day 8, indicating that the BSM/PCL/F127 composite scaffold provided a suitable microenvironment. Evaluation of the in vivo tumorigenicity of the scaffold with or without uUSCs 8 weeks after implantation showed that the 3 wt% BSM composite scaffold did not cause teratomas. The tumorigenicity of the 3 wt% BSM/F127 composite scaffolds was evaluated by implanting the scaffold into the subcapsular space of the kidneys in ICR mice (Fig. 8.2). BSM was used as the control. Scaffolds seeded with uUSCs were also tested. After 8 weeks, H&E staining showed no signs of tumor formation in any scaffold, with or without cells. Evaluation of the in vivo tumorigenicity of the scaffold with or without uUSCs, 8 weeks after implantation, showed that the 3 wt% BSM composite scaffold did not cause teratomas. With respect to tumorigenicity, this result supports the safety of the scaffold with or without cells for therapeutic applications.

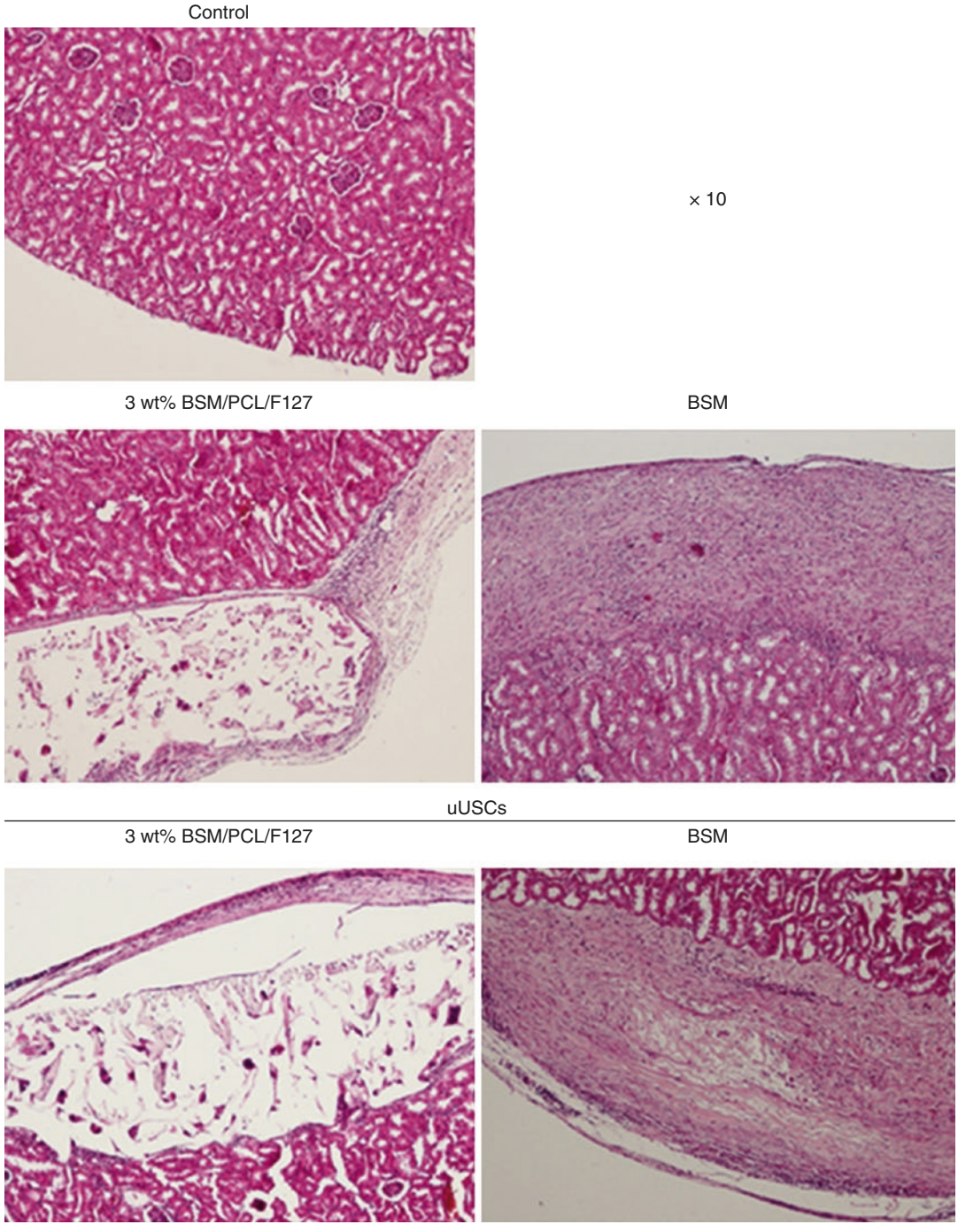

Fig. 8.2 Tumorigenicity analysis of the 3 wt% BSM composite scaffold. For this analysis, uUSCs were used. The BSM scaffold and a sham-operated kidney were used as controls. Control, non-scaffold-treated kidney

8.3 Bladder Regeneration Using Cell Transplantation

Regenerative medicine with selective cell transplantation may provide a means to create new functional bladder segments. The success of cell transplantation strategies for bladder reconstruction depends on the ability to efficiently use donor tissue and provide the right conditions for long-term survival, differentiation, and growth. Various cell sources have been explored for bladder regeneration. Cilento et al. [73] showed that native cells are preferable because they can be used without rejection. A simple method for harvesting bladder cell types from surgical specimens was used to generate normal human urothelial cell lines that could be reproducibly cultivated, passaged, and extensively expanded in serum-free medium. Immunostaining of the bladder epithelial cells with broadly reacting anti-cytokeratin antibodies and an anti-cytokeratin antibody specific to cytokeratin 7, a transitional cell marker, indicated that they expressed a stable epithelial phenotype during serial passaging. Low levels of immunostaining for E-cadherin and E-cadherin messenger ribonucleic acid by Northern blot analysis and strongly positive immunostaining for vimentin indicated that the uroepithelial cells express a non-barrier-forming phenotype under these culture conditions. However, when the urothelial cells were implanted subcutaneously into athymic mice on biodegradable synthetic polymers, they formed multilayered structures, suggesting that they retain the capability to differentiate in a living host. The urothelial cells proliferated in an epidermal growth factor-independent manner and expressed high levels of transforming growth factor-alpha and amphiregulin messenger ribonucleic acids, suggesting autocrine regulation of growth by epidermal growth factor-like factors. Cytogenetic analysis indicated that urothelial cells cultured for six passages possessed a normal chromosomal complement. These results demonstrate that primary cultures of autologous human bladder epithelial cells can be extensively expanded in vitro and, consequently, might be useful in cell transplantation strategies for genitourinary reconstruction. Amniotic fluid- and bone marrow-derived stem cells can also be used in an autologous manner and have the potential to differentiate into bladder muscle and urothelium [74–76]. Embryonic stem cells and uUSCs also have the potential to differentiate into bladder tissue [77, 78].

8.3.1 Mesenchymal Stem Cell

De Coppi et al. [74] set up a model of bladder acute necrotizing injury to test the efficacy of adult rat bone marrow mesenchymal stem cell (MSC) vs. fetal rat amniotic fluid MSC transplantation for the treatment of the impaired detrusor muscle contractility that develops when a cryoinjury is applied to the bladder wall. Impaired detrusor muscle contractility is part of the wound-healing process, which is characterized by SMC depletion, followed by SMC hyperplasia and tissue remodeling in the surviving bladder. At 30 days after transplantation, only a few fetal or adult MSCs gave rise to enteric or vascular SMCs, whereas most MSCs appeared incapable of specific differentiation. In vitro coculture experiments using SMCs with fetal or adult MSCs selectively labeled with distinct fluorochromes showed the presence of hybrid cells, suggesting that some MSCs can undergo cell fusion. Surprisingly, the major effect of rat bone marrow or amniotic fluid MSC transplantation seemed to be preventing the cryoinjury-induced hypertrophy of surviving SMCs. In this model, stem cell transplantation had a limited effect on SMC regeneration. Instead, it regulated post-injury bladder remodeling, possibly via a paracrine mechanism.

The potential risk of using diseased tissue as a source of cells for tissue engineering necessitates the investigation of other possible cell sources. One promising source is the bone marrow, which is a known source of stem cells for hematopoietic and mesenchymal lineages. Bone marrow-derived MSCs mobilize from the marrow in response to tissue damage and contribute to the normal regeneration process, and MSCs have been shown to localize in urinary bladders undergoing tissue regeneration after acellular bladder

augmentation in rats [79]. Shukla et al. [67] characterized bone marrow-derived MSCs from pigs and demonstrated their ability to differentiate into SMCs and utility for autologous augmentation cystoplasty. MSCs were isolated from pigs and analyzed for common markers of MSCs by flow cytometry, and SMC differentiation was assessed by immunoblotting. MSCs were isolated, genetically labeled, expanded in vitro, seeded onto SIS, and used for autologous bladder augmentation. Porcine MSCs are morphologically and immunophenotypically similar to human MSCs. Culturing MSCs at low density enhances their proliferation rates, whereas maintenance at confluence consistently induces differentiation into mature SMCs. Labeled MSCs grew on SIS for 1 week in vitro and survived a 2-week implantation as an autologous bladder augment in vivo. Some SMC label-positive cells with typical SMC morphology were detected; however, most cells were SMC label negative. Notably, many cells with a urothelial morphology stained positively for SMC markers. Porcine MSCs have properties similar to those of MSCs from other species and consistently undergo differentiation into mature SMCs in vitro under specific culture conditions. The addition of MSCs to SIS may enhance tissue regeneration in augmentation cystoplasty; however, they may not be significantly incorporated into smooth muscle bundles.

Anumanthan et al. [76] reported directed differentiation of bone marrow-derived MSCs into bladder urothelium for use as a source of pluripotent or multipotent progenitor cells. The epithelium was separated from the mesenchymal shells of embryonic day 14 rat bladders. MSCs were isolated from mouse femoral and tibial bone marrow, and heterospecific recombinant xenografts were created by combining embryonic rat bladder mesenchymal shells with the MSCs and grafting them into the renal subcapsular space of athymic nude mice. Grafts were harvested at time points of up to 42 days and stained for urothelial and stromal differentiation. Histological examination of xenografts comprising mouse MSCs and rat embryonic rat bladder mesenchyma yielded mature bladder structures with normal microscopic architecture and expression of proteins confirming functional characteristics. Specifically, the induced urothelium expressed uroplakin, a marker of urothelial differentiation. These differentiated bladder structures also showed appropriate alpha-smooth muscle actin staining. Finally, Hoechst staining of the xenografts revealed a nuclear architecture consistent with a mouse mesenchymal stem cell origin in the urothelium, supporting the differentiation of these cells. In the appropriate signaling environment, bone marrow-derived MSCs can undergo directed differentiation toward endodermal-derived urothelium and develop into mature bladder tissue within a tissue recombination model. This model serves as an important tool for the study of bladder development with a long-term goal of cell replacement therapy applications.

8.3.2 Embryonic Stem Cell

In 1981, embryonic stem (ES) cells were isolated from mice for the first time [80]. This major breakthrough revolutionized the field of developmental biology. ES cells are capable of prolonged self-renewal and differentiation, providing a tool to investigate the molecular mechanisms occurring during differentiation from the embryo to adult. ES cells are considered to be pluripotent and can differentiate into almost all cell types that arise from the three embryonic germ layers [81]. In vitro, these cells can differentiate into multiple embryonic and adult cell types but rarely cells of endodermal lineage [82]. In contrast, differentiation of ES cells in an in vivo environment shows their full developmental potential. Whether stem and/or progenitor cells exist within the bladder is unknown, but hypothetically, they should exist. Identification of stem cells within the vast population of cells in the bladder would be challenging, especially without bladder-specific stem/progenitor cell markers. Oottamasathien et al. [77] determined the specific mesenchymal to ES cell ratios necessary to promote organ-specific differentiation while completely suppressing teratomatous growth. The embryonic mesenchyme is well established as an inductive

tissue that dictates organ-specific programming of epithelial tissues, and this study showed that embryonic bladder mesenchyme can also drive ES cell differentiation toward endodermal-derived urothelium. These approaches allow us to capture specific stages of stem cell differentiation and better define stem cell hierarchies.

8.3.3 Urine-Derived Stem Cell

Urine-derived stem cells (USCs) consistently expressed MSC/pericyte markers and some key cell surface markers but no hematopoietic stem cell markers (except for MHC-1), endothelial markers (CD31), or human leukocyte antigen (locus) DR (HLA-DR) [83]. Compared to other MSCs, USCs have several advantages: (1) they can be collected using a simple, low-cost, safe, noninvasive procedure; (2) they display telomerase activity, and, thus, they are able to generate more cells; and (3) they can efficiently differentiate into SMCs, UCs, and endothelial cells. Chun and Kim et al. [70] investigated whether cells isolated from the upper urinary tract (UTCs) possess stem cell characteristics and could be used as an alternative cell source for patients with bladder cancer. Current tissue engineering approaches for urologic tissue regeneration require invasive tissue biopsies to obtain autologous cells, and these procedures are associated with various potential complications, such as donor site morbidity. Recently, cells isolated from voided urine (VUCs) have been proposed as an alternative cell source for urologic tissue engineering. However, VUCs should not be used in patients with bladder cancer, because the voided urine sample could contain malignant cells. In the study, urine samples were collected from the upper urinary tract of four male patients with bladder cancer using a ureteral catheter. The samples were centrifuged, and the cell pellets were plated for primary culture. The cells were analyzed for the number of colony-forming units, proliferation rate, cytogenetics, stem cell characteristics, and tumorigenicity, and the results were compared to those of VUCs collected from three healthy men. The UTCs were able to form colonies, had a greater proliferation rate than the VUCs, and had a normal karyotype. The UTCs possessed stem cell characteristics (expression of CD44+, CD73+, CD90+, CD105+, and SSEA4+) and expressed several markers of the urothelial, smooth muscle, and endothelial cell lineages. The UTCs did not form teratomas when implanted into the subcapsular space of a mouse kidney. Since the UTCs possessed stem cell characteristics, they could potentially be an alternative cell source for urologic tissue regeneration in patients with bladder cancer.

8.3.4 Induced Pluripotent Stem Cell

Induced pluripotent stem cells (iPSCs) are naturally programmed to divide continuously and remain undifferentiated. Although these cells can give rise to ectodermal, mesodermal, or endodermal cell lineages, a significant risk of teratoma exists. Any undifferentiated iPSCs placed in the body might continue to divide in an uncontrolled manner, forming tumors. In addition, it takes a long time (4 months) to derive and characterize iPSCs from an individual. Furthermore, the low efficiency of differentiation, genetic abnormalities, and high cost prohibit their clinical applicability. Despite this, a few studies of ESCs or iPSCs for bladder tissue engineering have been reported. Frimberger et al. [76] reported that human embryoid body-derived stem cells showed improved migration in the presence of mature human bladder SMCs and urothelial cells. In addition, Moad et al. [77] reported the generation of human iPSCs derived from normal and aging human urinary tract tissue. These iPSCs underwent bladder differentiation more efficiently than skin-derived iPSCs, as shown by the expression of urothelial-specific markers (uroplakins, claudins, and cytokeratin) and stromal smooth muscle markers (alpha-smooth muscle actin, calponin, and desmin), indicating the importance of organ-specific iPSCs for tissue-specific studies. Immobilized

cell lines are not suitable for bladder regeneration due to safety concerns. Therefore, multipotent adult stem cells are currently used in bladder repair and reconstruction. Of particular interest is the paper published by Xue et al. [84], in which they describe a practical method to generate human iPSCs from USCs under feeder-free, virus-free, serum-free conditions without the c-MYC oncogene. The authors showed that this approach could be applied in a large population with different genetic backgrounds. USCs are easily accessible and exhibit high reprogramming efficiency, offering several advantages over other cell types used for iPSC generation. Using the approach described in this study, the authors generated 93 iPSC lines from 20 donors with diverse genetic backgrounds. The nonviral iPSC bank containing these cell lines is a valuable resource for iPSC research, facilitating future applications of human iPSCs. Table 8.1 shows a comparison of the various stem cell types used in bladder repair studies.

Table 8.1 Comparison of the various stem cell types used for bladder repair

Cell type/parameter	BMSCs	ASCs	USCs	ESC/iPSCs	Bladder SMCs and UCs
Self-renewal and expansion capability	Limited, PD ~30		High, PD 60–70	Very high, PD >200	Limited, PD <30
Multi-lineage differentiation capability	Multipotent but mainly limited to mesodermal cell lineages	Similar to BMSCs	Multipotent differentiation potential	Pluripotent (can generate all lineages)	None
Urothelial and endothelial differentiation capability	Low (<10%)	Low (10%)	High (60–85%)	Low	
Telomerase activity (TA)/ telomere length	Cannot be detected	Cannot be detected	Up to 75% of USC clones possess TA and relatively long telomeres	Possess TA and long telomeres	None
Harvesting approach	Invasive	Invasive	Noninvasive, simple, low cost, safe	Invasive to harvest somatic cells to generate iPSCs	Invasive
Pure stem cell isolation	Difficult	Difficult	Very easy	Easy	None
Number of stem cells harvested	MSC/10^4 bone marrow stromal cells in newborns, 1MSC/10^6		100–140 USC clones/24 h urine from adults		Unknown
Angiogenic trophic factors	Yes	Yes	Yes	Unknown	Moderate
Immunomodulatory properties	Yes	Yes	Yes	Unknown	Unknown
Rejection after implantation	No rejection as allogenous or xenogenous cells (e.g., human BMSCs or USCs) when implanted in rodent, rabbit, or canine models			Likely to be rejected	No rejection as autogenous cells
Oncogenic potential	No	No	No	Yes	None
Clinical trial utility	Potential	Potential	Potential	Safety concern	Yes

ASC adipose-derived stem cell, *BMSC* bone marrow-derived mesenchymal stromal cell, *ESC* embryonic stem cell, *iPSC* induced pluripotent stem cell, *MSC* mesenchymal stem cell, *PD* population doubling, *SMC* smooth muscle cell *UC* urothelial cell, *USC* urine-derived stem cell

8.4 Tissue Engineering Approach for Bladder Regeneration

Even in multiple studies, implantation of biomaterials without cells into the bladder has shown some promising results, especially the urothelial layer which was able to regenerate normally; however the regeneration of muscle layer was not fully developed [55, 62, 64, 66, 85]. Therefore, many investigators preferred tissue engineering approach (grafting biomaterials seeded with cells) for bladder tissue regeneration. In the early stage of investigation, synthetic polymer seeded with autologous cells was the most commonly used approach. The autologous urothelial and muscle cells can be expanded in vitro, seeded onto polymer scaffolds, and allowed to attach and form sheets of cells. The resulting cell-polymer scaffold can then be implanted in vivo. Histological analysis showed that viable cells were able to self-assemble back into their respective tissue types and retain their native phenotypes [86]. Synthetic polymer fibers of polyglycolic acid can serve as both a scaffold and delivery vehicle for the implantation of rabbit uroepithelial cells into athymic host animals. The polymers, which slowly degrade in vivo, allow the urothelial cells to survive at the implant site. In the abovementioned study [79], the authors demonstrated that polyglycolic acid polymers support the proliferation of rabbit urothelial cells in situ and can serve as a malleable substrate for the creation of new urological structures that replace the degrading polymer fibers. They also showed that when implanted on polyglycolic acid fibers, human urothelial and bladder muscle cells form new urological structures composed of both cell types. Human cell-polymer xenografts can be recovered from host animals at extended time points after implantation. These data suggest the feasibility of using polyglycolic acid polymers as substrates for the creation of human urothelial and muscle grafts for genitourinary reconstruction. These experiments demonstrated, for the first time, that composite-layered tissue-engineered structures could be created de novo.

8.4.1 Animal Models

Jayo et al. compared the in situ cellular responses to two biopolymer implants, a polylactic-co-glycolic acid-based biodegradable mesh scaffold with autologous urothelial cells and SMCs (construct) and a PLGA-based biodegradable mesh scaffold without cells (scaffold), in a canine model of augmentation cystoplasty [87]. Healing events were correlated with urodynamic assessments. Construct implants regenerated baseline urodynamics as early as 4 months after implantation. In contrast, following scaffold implantation, urodynamics failed to return to baseline by study termination (9 months). The functional differences elicited by the construct and scaffold implants were correlated with structural differences in the neo-tissues. The construct stroma had greater vascularization, with gently folded, interwoven connective tissue elements. Conversely, the scaffold stroma was dense, with haphazardly organized connective tissue. The urothelium was regenerated in response to both construct and scaffold implantation. However, only the construct urothelium had normal stroma, well-developed detrusor, and abundant alpha-smooth muscle actin cell staining at early time points, leading to a structurally and functionally complete bladder wall at 9 months. They concluded that early cellular and stromal events distinguished the healing processes that led to bladder wall regeneration or repair. Construct implants containing cells elicited early healing processes that culminated with the regeneration of complete mucosal and muscular components. In contrast, the response to scaffold implantation was consistent with reparative healing, i.e., mucosal growth, but incomplete tissue layer development. These independent studies demonstrate that cells are necessary to improve bladder function when a large bladder tissue implant is required.

The utility of allogenic bladder submucosa seeded with cells as a biomaterial for bladder augmentation was investigated by Yoo et al. in 1998 [62]. Partial cystectomies were performed in ten beagle dogs. Both urothelial and SMCs were harvested from five animals and expanded

separately. Allogenic bladder submucosa obtained from sacrificed dogs was seeded with muscle cells on one side and urothelial cells on the opposite side. All beagles underwent cruciate cystotomies on the bladder dome. Augmentation cystoplasty was performed with the cell-seeded allogenic bladder submucosa in five animals and unseeded allogenic bladder submucosa in five animals. The augmented bladders were retrieved 2 and 3 months after augmentation. Bladders augmented with the cell-seeded allogenic bladder submucosa showed a 99% increase in capacity compared to bladders augmented with the cell-free allogenic bladder submucosa, which showed only a 30% increase in capacity. All dogs showed normal bladder compliance, as evidenced by urodynamic studies. Histologically, all retrieved bladders contained a normal cellular organization consisting of a urothelial-lined lumen surrounded by submucosal tissue and smooth muscle. Immunocytochemical analyses confirmed the urothelial and muscle cell phenotypes and showed the presence of nerve fibers. In summary, these matrices can function as vehicles for partial bladder regeneration, and no relevant antigenicity is evident.

It has been known for decades that the bladder can regenerate extensively over free grafts, as the urothelium has a high reparative capacity [88]. In their study, de Boer et al. investigated the spatiotemporal changes in the RNA and protein expression of growth factors and their receptors by in situ hybridization and immunocytochemistry during regeneration after acute injury of mouse urothelium. These expression data were well correlated with the changes in cell morphology and proliferation. Except for enhanced muscular transforming growth factor-beta 1 (TGF-beta 1) and TGF-beta type 2 receptor expression, the changes in the expression patterns of growth factors or receptors were confined to the urothelium. Increased mucosal RNA expression of insulin-like growth factor-2 (IGF-2) and particularly type 1 IGF receptor, as well as fibroblast growth factor-1 (FGF-1) but not FGF-2, coincided with reepithelialization and urothelial proliferation. High levels of urothelial TGF-beta 1 RNA and protein expression were associated with reepithelialization and differentiation. In addition, TGF-beta type 2 receptor protein expression was enhanced in the urothelium. Platelet-derived growth factor-A (PDGF-A) RNA was constitutively expressed in the mucosa, but expression decreased in the reepithelialization phase. These data are consistent with the notion that urothelial regeneration can be achieved through paracrine or autocrine mechanisms via urothelium-derived growth factors. The observation of analogous growth factor RNA expression patterns in regenerating skin epidermis suggests a more general growth factor-regulated mechanism for epithelial regeneration.

Bladder muscle tissue is less likely to regenerate normally. Both urothelial and muscle ingrowth are believed to be initiated from the edges of the normal bladder toward the region of the graft [89]. Regeneration of smooth muscle appears to take place within the fibrous tissue characteristically found when biodegradable collagen/Vicryl prosthesis is used to repair full-thickness defects in the rabbit urinary bladder. The question of whether the central smooth muscle was generated via myoblastic differentiation within the fibrous tissue or arose from healthy preexisting detrusor muscle was addressed by serial sectioning and specific staining. Only in situ transmutation, or differentiation, explains the observed morphology, and the results strongly suggest that the central smooth muscle was regenerated from within the repair area.

However, contracture or resorption of the graft is usually evident. The inflammatory response to the matrix may contribute to resorption of the free graft. It was hypothesized that building 3-D constructs in vitro prior to implantation might facilitate the eventual terminal differentiation of the cells after implantation while minimizing the inflammatory response toward the matrix, thus avoiding graft contracture and shrinkage. A study in dogs demonstrated a major difference between matrices with autologous cells (tissue-engineered matrices) and those without cells [62]. Matrices implanted with cells retained most of their implanted diameter, whereas matrices implanted without cells showed graft contraction and shrinkage. The histomorphology demonstrated a

marked paucity of muscle cells and a more aggressive inflammatory reaction in the matrices implanted without cells.

To better address the functional parameters of tissue-engineered bladders, a canine animal model was designed that required a subtotal cystectomy and subsequent replacement with a tissue-engineered organ [90]. Cystectomy-only and non-seeded controls maintained average capacities of 22% and 46% of the preoperative values, respectively. In contrast, an average bladder capacity of 95% of the original precystectomy volume was achieved in the cell-seeded tissue-engineered bladder replacements. These findings were confirmed radiographically. Histologically, the non-seeded scaffold bladders presented a pattern of normal urothelial cells with a thickened fibrotic submucosa and a thin layer of muscle fibers. The retrieved tissue-engineered bladders showed a normal cellular organization, consisting of a trilayer of urothelium, submucosa, and muscle. These studies, performed with PGA-based scaffolds, have been repeated by other investigators, and similar results in long-term studies of large numbers of animals have been reported [85, 87]. Jayo et al. [85] evaluated bladder regeneration following 80% cystectomy and augmentation using a synthetic biopolymer with autologous urothelial and SMCs (autologous neo-bladder augmentation construct [construct]) or autotransplantation of native bladder (reimplanted native urinary bladder [reimplant]) in canines. Voiding function, urodynamic assessment, and neo-organ capacity-to-body-weight ratio (C/BW) were assessed longitudinally for 24 months following trigone-sparing augmentation cystoplasty in juvenile canines. Within 30 days postimplantation, hematology and urinalysis returned to baseline. Both the constructs and reimplants yielded neo-organs with statistically equivalent urodynamics and histology. Linear regression analysis of C/BW showed that the constructs regained baseline slope and continued to adapt with animal growth. Constructs and reimplants regained and maintained native bladder histology by 3 months, capacity at 3–6 months, and compliance by 12–24 months. Furthermore, the construct C/BW demonstrated the ability of the regenerated bladder to respond to growth regulation. However, not all scaffolds perform well when used to replace a large portion of the bladder. In a study using SIS for subtotal bladder replacement in dogs, both the unseeded and cell-seeded experimental groups showed graft shrinkage and poor results [91]. In the study, 22 male dogs had a 90% partial cystectomy and were divided into three groups. At 1 month after cystectomy, dogs in the unseeded (n = 6) and seeded (n = 6) groups received a bladder augmentation with a corresponding SIS graft. The dogs in the surgical control group (n = 10) received no further surgery. All dogs were evaluated before and after surgery with blood chemistry, urine culture, intravenous urography, cystogram, and cystometrogram. After surgery (at 1, 5, and 9 months), the bladders were examined by routine histology and immunohistochemistry. All 22 dogs survived the subtotal cystectomy, and 18 survived to the end of their intended survival period. One dog in the seeded group died 1 month after augmentation due to a bladder perforation caused by a large piece of incompletely absorbed SIS. Three other dogs (two in the unseeded group and one in the seeded group) died within 2 months after augmentation due to bladder obstruction by stones. Unseeded and seeded SIS grafts showed moderate to heavy adhesion and graft shrinkage, and some had bone and calcification at the graft site. In both groups, histology showed limited bladder regeneration. Interestingly, dogs in the control group at 1 month after cystectomy (when the seeded and unseeded groups received their augmentations) had severely shrunken bladders and histologically showed severe inflammation, fibroblast infiltration, and muscle hypertrophy. These results verify the subtotal cystectomy model. The use of seeded or unseeded SIS in a subtotal cystectomy model does not yield the same quality and quantity of bladder regeneration observed in the 40% noninflammatory cystectomy model. This study provides important insights into the process of regeneration in a severely damaged bladder. These results led us to reevaluate the critical

elements required for complete bladder replacement using tissue engineering.

The type of scaffold used is critical for the success of tissue engineering-based bladder replacement. The use of bioreactors, wherein mechanical stimulation is initiated at organ production, has also been proposed as an important parameter for success [92, 93]. Farhat and Yegar [92] reported that mechanical stimulation may have a role in urinary bladder tissue engineering. Currently, tissue engineering of the urinary bladder relies on biocompatible scaffolds that deliver biological and physical functionality with negligible immunogenic or tumorigenic risks, and recent research suggests that autologous cells propagated in culture and seeded on scaffolds prior to implantation improve clinical outcomes. In addition, as normal urinary bladder development in utero requires regular filling and emptying, current research suggests that bladders constructed in vitro may also benefit from regular mechanical stimulation. Such stimulation appears to induce favorable cellular changes, proliferation, and the production of structurally suitable extracellular matrix (ECM) components that are essential for the normal function of hollow dynamic organs. To mimic in vivo urinary bladder dynamics, tissue bioreactors that imitate the filling and emptying of a normal bladder have been devised. A "urinary bladder tissue bioreactor" that is able to recapitulate these dynamics while providing a cellular environment that facilitates the normal cell-cell and cell-matrix interactions may be necessary to successfully engineer bladder tissue. Validation of a urinary bladder tissue bioreactor that permits careful control of physiological conditions will generate broad interest from researchers in urinary bladder physiology and tissue engineering. A similar study was conducted by Bouhout et al. [86], showing a bladder substitute that was reconstructed in a physiological pressure environment. Bladder reconstruction by enterocystoplasty or with bioengineered substitutes is still associated with complications, which led us to develop an autologous vesical equivalent (VE). This model has already proven its structural conformity. The current challenge is to reconstruct our model in a more physiological environment, with the use of a bioreactor that mimics the dynamics of bladder filling and emptying, to acquire the proper physiological properties. In our model, fibroblasts and urothelial cells were evolved in a 3-D culture to obtain a reconstructed VE. This was then cultured in our bioreactor, which delivers a cyclic pressure increase up to 15 cm H_2O, followed by a rapid decrease, to achieve a dynamically cultured VE (dcVE). The dcVE was characterized by histology and immunofluorescence and compared to the characteristics of statically cultured VE. Mechanical resistance was evaluated by uniaxial tensile tests, and permeability was measured with 14C-urea. Compared to our static model, the dynamic model led to a urothelium profile similar to that of native bladder. Permeability analysis showed a profile comparable to that of native bladder, coinciding with the basal cell organization in the dcVE, and appropriate resistance for suturing and handling was also shown. This new alternative method offers a promising avenue for regenerative medicine. It is distinguished by its autologous character and efficiency as a urea barrier. These properties could significantly reduce inflammation, necrosis, and possibly rejection.

8.4.2 Human Models

Clinical trials of engineered bladder tissue for cystoplasty reconstruction began in 1998. The first was a small pilot study of seven patients using either a cell-seeded collagen scaffold (with or without omentum coverage) or a combined PGA-collagen cell-seeded scaffold with omental coverage. The patients who underwent reconstruction with the engineered bladder tissue created with the PGA-collagen cell-seeded scaffolds with omental coverage showed increased compliance, decreased end-filling pressure, increased capacity, and longer dry periods over time [12]. In this study, the outcomes were measured by serial urodynamics, cystograms, ultrasounds, bladder biopsies, and serum analyses, and the average follow-up was 46 months (range, 22–61 months). Postoperatively, the mean bladder leak

point pressure decreases at capacity, and the greatest increase in volume and compliance was observed in the composite engineered bladders with an omental wrap (56%, 1.58-fold and 2.79-fold, respectively). Bowel function returned promptly after surgery. No metabolic consequences were noted, urinary calculi did not form, mucus production was normal, and renal function was preserved. The engineered bladder biopsies showed an adequate structural architecture and phenotype. Based on these results, engineered bladder tissues created with autologous cells seeded on collagen-polyglycolic acid scaffolds and wrapped in omentum after implantation can be used in patients who require cystoplasty. Although these results are promising since they show that engineered tissues can be implanted safely, it is just a start in terms of accomplishing the goal of engineering fully functional bladders. This was a limited clinical experience, and the technology is not yet ready for wide dissemination; further experimental and clinical studies are required, and phase 2 studies have been completed.

8.4.3 Neo-urinary Conduits

From the aforementioned and the urethral studies, it is evident that the use of cell-seeded matrices is superior to non-seeded matrices for the creation of engineered bladder tissues. Although advances have been made in bladder tissue engineering, many challenges remain. Much of the current research is aimed at the development of biologically active, "smart" biomaterials that may improve tissue regeneration. Similar engineering techniques are now being used in patients with bladder cancer who are having engineered urinary conduits implanted after cystectomy [94]. Muscle-invasive and recurrent non-muscle-invasive bladder cancers have been traditionally treated with a radical cystectomy and urinary diversion. The urinary diversion is generally accomplished through the creation of an incontinent ileal conduit, continent catheterizable reservoir, or orthotopic neo-bladder utilizing the small or large intestine. While radical extirpation of the bladder is often successful from an oncological perspective, there is significant morbidity associated with enteric interposition within the genitourinary tract. Therefore, there is a great opportunity to decrease the morbidity associated with the current surgical management of bladder cancer by utilizing novel technologies to create a urinary diversion without the intestine. Clinical trials using neo-urinary conduits (NUC) seeded with autologous SMCs are currently in progress and may offer a significant surgical advance by eliminating the complications associated with the use of gastrointestinal segments in urinary reconstruction, simplifying the surgical procedure, and greatly facilitating recovery from cystectomy. A conduit from the ureters to the skin surface addresses the current standard of care while simplifying the surgical procedure, and it may also improve patient outcomes. The NUC created by Tengion serves as a template to catalyze the regeneration of native-like urinary tissue that can connect the ureters to the skin surface. To ensure native urinary tissue regeneration, a biocompatible, biodegradable scaffold with an extended history of safety and clinical utility is necessary. The broadly used PLGA scaffold can enhance tissue regeneration and promote neo-tissue integration when properly seeded with SMCs. The NUC construct has two principal components. The first is the biomaterials. The NUC scaffold is composed of a PGA polymer mesh fashioned into the required tubular shape and coated with a 50/50 blend of PLGA copolymer. The specific structural parameters of the construct can be modified during the surgical procedure according to the patient's needs. The choice of well-established, synthetic, degradable biopolymers reflects the same requirements for reliability and reproducibility inherent in the choice of these polymers for applications in other bladder-related neo-organs. The second component is the cells. Autologous SMCs sourced from bladder or non-bladder tissue may be applied for NUC construction. Based on the successful outcomes in a porcine cystectomy model, Tengion has initiated phase I clinical trials of NUC constructs in human patients requiring urinary diversion. This phase I study, "Incontinent Urinary Diversion Using an

Autologous Neo-Urinary Conduit" (http://www.clinicaltrials.gov/ct2/show/NCT01087697), is currently recruiting patients, with the objective of implanting up to ten patients by the end of 2012. The objective of the study is to evaluate if NUC constructs made using autologous adipose-derived SMCs in combination with defined degradable biomaterial scaffolds can form a functional conduit to safely facilitate passage of urine from the kidneys subsequent to radical cystectomy. Primary outcome indices over a 12-month postimplantation period include structural integrity and conduit patency. CT scans will be used to demonstrate that urine flows safely through the NUC construct. Additional measures of primary outcomes up to 12-month postimplantation include evaluation of any product- or procedure-related adverse events. Similarly, secondary outcome indices will include analysis of NUC structural integrity and patency over a 12–60-month postimplantation period. CT scan and renal ultrasound will be applied to demonstrate that urine flows safely through the NUC construct up to 60 months after implantation. Procedural- and product-related adverse events will also be monitored up to 60 months after implantation. Finally, the overall safety of the NUC construct will be assessed by evaluation of nonproduct-/procedural-related adverse events and patient vital signs.

Stem cells derived from fat can be differentiated into smooth muscle for use in the conduits, thus avoiding the use of bladder cells from bladder cancer patients [95]. Basu et al. [88] described the isolation and characterization of SMCs from porcine adipose tissue and peripheral blood that are phenotypically and functionally indistinguishable from bladder-derived SMCs. In a preclinical Good Laboratory Practice study, we demonstrated that autologous adipose- and peripheral blood-derived SMCs can be used to seed synthetic, biodegradable, tubular scaffold structures and that implantation of these seeded scaffolds into a porcine cystectomy model leads to successful de novo regeneration of a tubular neo-organ composed of urinary-like neo-tissue that is histologically identical to native bladder. The ability to create urologic structures de novo from scaffolds seeded with autologous adipose- or peripheral blood-derived SMCs will greatly facilitate the translation of urologic tissue engineering technologies into clinical practice.

8.4.4 Kyungpook National University Experiences

In a very important study, Lee et al. [96] investigated the synergistic effect of human USCs and a surface-modified composite scaffold for bladder reconstruction in a rat model. The composite scaffold (PCL/F127/3 wt% BSM) was fabricated using an immersion precipitation method, and heparin was immobilized on the surface via covalent conjugation. A PCL pellet/Pluronic F127 powder mixture (95/5 [w/w]) was dissolved in tetraglycol (12 wt%), and BSM powder was evenly mixed with the polymer solution. The mixed solution was poured into a polytetrafluoroethylene mold (70 × 70 × 0.4 μL) and then immersed in water for 1 h at room temperature. After additional washing and vacuum drying, the PCL/F127/BSM composite scaffold was sterilized with ethanol. The PCL/F127/3 wt% BSM composite scaffold exhibited significantly enhanced hydrophilicity, the surface was easily immobilized, and there was no evidence of teratoma formation in vivo [1]. Basic fibroblast growth factor (bFGF) was loaded onto the heparin-immobilized scaffold by a simple dipping method. bFGF has been shown to stimulate the proliferation and survival of both SMCs and urothelial cells [97], suggesting the benefit of bFGF-loaded scaffolds for urological tissue engineering applications. To fabricate a scaffold for bFGF delivery, the authors covalently conjugated heparin to the surface of a scaffold to form a heparin-immobilized scaffold, which was then loaded with bFGF (scaffold$^{\text{heparin-bFGF}}$) [98]. Urine samples from the upper urinary tract were obtained from a 52-year-old female patient. The urine samples (100 mL each) were centrifuged, and the cell pellets were washed with PBS. The cells were cultured in a mixture of keratinocyte serum-free medium and progenitor cell medium (Gibco-Invitrogen, Grand Island, NY, USA) in a

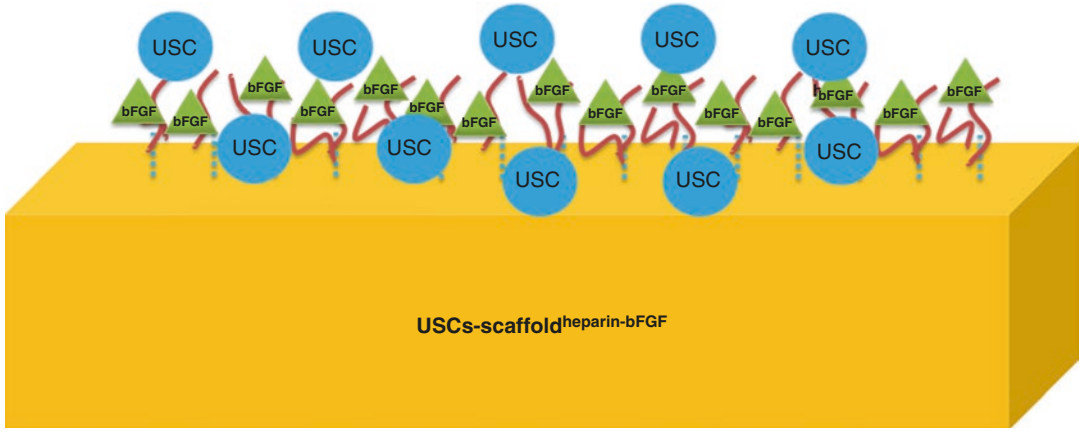

Fig. 8.3 Schematic diagram of the USC-seeded composite scaffold$^{heparin\text{-}bFGF}$ graft. *USC* urine-derived stem cell, *bFGF* basic fibroblast growth factor

1:1 ratio. USCs have been proposed as an alternative stem cell source for urological tissue reconstruction since they have mesenchymal stem cell characteristics and have the capacity to differentiate into a variety of urological cell lineages [78]. Figure 8.3 shows a schematic diagram of the USC-seeded composite scaffold$^{heparin\text{-}bFGF}$ graft. Twenty-five rats were divided into five groups: (1) control (sham operated), (2) partial cystectomy group (an ~40% defect was created in the dome of the bladder wall), (3) scaffold (an unmodified scaffold was attached after partial cystectomy), (4) scaffold$^{heparin\text{-}bFGF}$ (the heparin-immobilized bFGF-loaded scaffold was attached after partial cystectomy), and (5) USC-scaffold$^{heparin\text{-}bFGF}$ (scaffold$^{heparin\text{-}bFGF}$ combined with 1×10^4 USCs was attached after partial cystectomy) groups. The single-layer scaffold (disk shaped, 6 mm diameter) was sutured as a patch onto the bladder defect with 7-0 Vicryl sutures. The omentum was loosely wrapped over the graft and fixed with fibrin glue (Greenplast; Green Cross, Seoul, Korea) (Fig. 8.4). In maximal bladder capacity and compliance analyses at 8 weeks postoperation, the USC-scaffold$^{heparin\text{-}bFGF}$ group showed significant functional improvement (2.34 ± 0.25 mL and 55.09 ± 11.81 μL/cm H_2O) compared to the other groups (2.60 ± 0.23 mL and 56.14 ± 9.00 μL/cm H_2O, control group; 1.46 ± 0.18 mL and 34.27 ± 4.42 μL/cm H_2O, partial cystectomy group; 1.76 ± 0.22 mL and 35.62 ± 6.69 μL/cm H_2O, scaffold group; and 1.92 ± 0.29 mL and 40.74 ± 7.88 μL/cm H_2O, scaffold$^{heparin\text{-}bFGF}$ group; Table 8.2). In the histological and immunohistochemical analyses, the USC-scaffold$^{heparin\text{-}bFGF}$ group showed pronounced, well-differentiated, and organized smooth muscle bundle formation, a multilayered and pan-cytokeratin-positive urothelium, and high condensation of the submucosal area. The implanted scaffolds were not visible in the tissue sections because they were dissolved by xylene during specimen processing. The histological features of the implanted grafts showed that the regenerated portions of the bladders in the USC-scaffold$^{heparin\text{-}bFGF}$ group exhibited pronounced smooth muscle bundles, a multilayered urothelium, condensed submucosa layer formation, and restored bladder volume. These anatomical reconstructions are essential for functional compliance. However, the other scaffold groups showed only weak SMC bundles, a thin urothelium, and loose submucosa regeneration at the graft. These results suggest that the seeded USCs contributed to tissue regeneration. While the seeded cells were only expected to survive for 2 weeks in vivo [99], these exogenous cells were more effective for bladder regeneration than the cells recruited from the surrounding host tissues or circulating blood flow. Although the USC regenerative mechanism was not determined in this study, based on previous reports, regeneration is presumed to result from

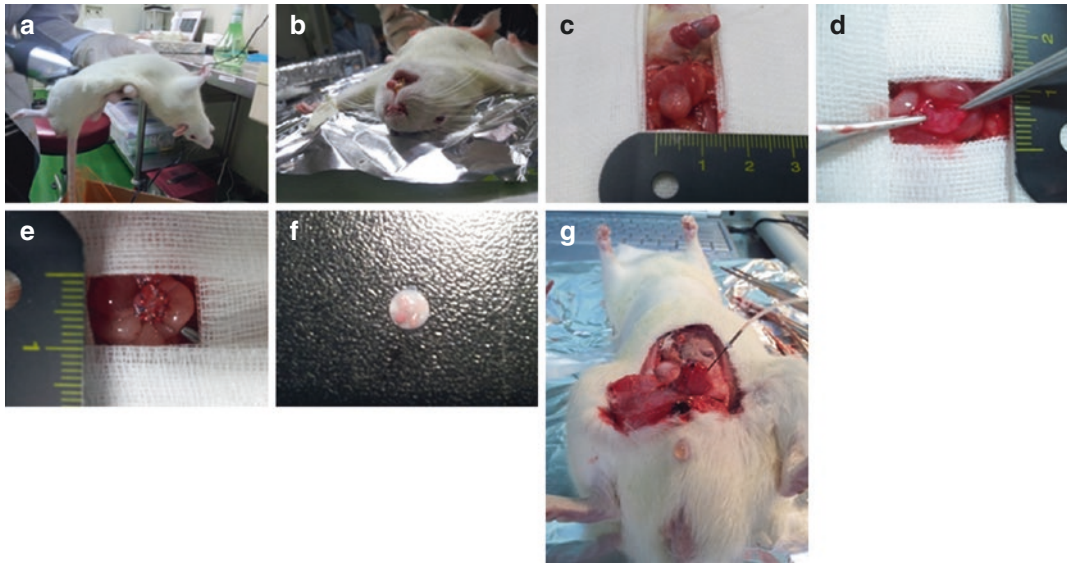

Fig. 8.4 Bladder reconstruction procedure using the USC-scaffold$^{heparin\text{-}bFGF}$. (**a**) Rats were anesthetized with an intraperitoneal injection of ketamine (40 mg/kg) and Rompun (10 mg/kg), with periodic supplementation as needed. (**b**) The rats were placed on the operating board in a supine position in laminar flow. (**c**) After shaving and sterilization with povidone iodine scrub over the suprapubic region, the abdomen was opened through a 4-cm longitudinal midline incision, and the urinary bladder was exposed. (**d**) The upper half of the bladder (dome and upper portion) was transected and removed with scissors. (**e**) The single-layer scaffold (disk form; diameter, 6 mm) was sutured as a patch onto the defect with 7-0 Vicryl sutures. (**f**) Representative picture of the scaffold and USC-scaffold$^{heparin\text{-}bFGF}$. (**g**) A urodynamic study (filling cystometry). The bladder was filled with PBS, and maximal capacity was defined as the minimum infusion volume that triggered urine leakage. Compliance was defined as the maximal capacity/(pressure that triggered leakage)—(baseline pressure). *USC* urine-derived stem cell, *bFGF* basic fibroblast growth factor

Table 8.2 Measurement of cross-sectional area and urodynamics, including maximal bladder capacity and compliance

	Control	Partial cystectomy	Scaffold	Scaffold$^{heparin\text{-}bFGF}$	USC-scaffold$^{heparin\text{-}bFGF}$
Cross-sectional area, mm^2 (mean ± SD)	16.85 ± 1.21	7.87 ± 1.37	9.67 ± 0.87	11.19 ± 0.87	15.71 ± 1.34
Maximal bladder capacity, mL (mean ± SD)	2.60 ± 0.23	1.46 ± 0.18	1.76 ± 0.22	1.92 ± 0.29	2.34 ± 0.25
Compliance, μL/cm H_2O (mean ± SD)	56.14 ± 9.00	34.27 ± 4.42	35.62 ± 6.69	40.74 ± 7.88	55.09 ± 11.81

USC urine-derived stem cell, *bFGF* basic fibroblast growth factor

the paracrine effects of trophic factors secreted from the USCs [99]. Thus, the transplanted stem cells influenced the surrounding host cells, resulting in improved cell migration and differentiation into the target cell type. The USC-seeded scaffold$^{heparin\text{-}bFGF}$ exhibited significantly increased bladder capacity, compliance, regeneration of smooth muscle tissue, multilayered urothelium, and condensed submucosa layers in the study. Based on these results, the USC-scaffold$^{heparin\text{-}bFGF}$ would be ideal for bladder reconstruction.

8.4.5 Whole Bladder Reconstruction

An interesting study was published by Hoogenkamp et al [100]. They showed that scaffolds made from molecularly defined biomaterials are instrumental in the regeneration of tissues but are generally confined to small flat patches and do not comprise the whole organ. In this study, a simple, one-step casting method was developed to produce a seamless, large, hollow, collagen-based scaffold, mimicking the shape of the whole bladder with integrated anastomotic sites for ureters and the urethra. This hollow bladder scaffold is highly standardized with uniform wall thickness and a unidirectional pore structure to facilitate cell infiltration in vivo. Human and porcine bladder urothelial cells and SMCs were able to attach to the scaffold and maintained their phenotypes in vitro. The closed luminal side and the porous outside of the scaffold facilitated the formation of a urothelial lining and infiltration of SMCs, respectively. The cells aligned according to the scaffold template. The technology used is highly adjustable in terms of shape, size, and materials and could be used as a starting point for research aimed at an off-the-shelf medical device for neo-bladders.

Tissue engineering of the bladder may become a reality in the future. It is important to design a preclinical study using the best predictive model with an adequate disease background to mimic the clinical situation. For bladder tissue engineering, large animals with diseased bladders appear to represent the best experimental model, which should aid in the development of clinically applicable tissue-engineered bladder augmentation or replacement with satisfactory long-term outcomes.

8.5 Conclusions and Perspectives

To prevent the problematic outcomes of bowel-based bladder reconstructive surgery, such as prosthetic tumors and systemic metabolic complications, the research focus has switched from regenerating and strengthening the failing organ or building an organ replacement in the 1990s to regenerative medicine and tissue engineering. Various types of acellular matrices, naturally derived materials, and synthetic polymers have been used for either unseeded (cell-free) or autologous cell-seeded tissue engineering scaffolds. Different categories of cell sources, from autologous differentiated urothelial cells and SMCs to natural or laboratory-derived stem cells, have been tested to obtain suitable “cell-seeded” templates. The current clinically validated bladder tissue engineering approaches essentially consist of augmentation cystoplasty in patients suffering from poorly compliant neuropathic bladder. There have been no clinical applications of whole tissue-engineered neo-bladder for radical-reconstructive surgical treatment of bladder malignancies or chronic inflammation due to vesical coarctation. The reasons why bladder tissue engineering has not yet been clinically applied include the risk of graft ischemia and subsequent fibrous contraction and perforation. The generation of a graft vascular network (vasculogenesis), together with the promotion of surrounding vessel sprouting (angiogenesis), could allow an effective graft blood supply and avoid serious, ischemia-related complications.

Current research suggests that the use of biomaterial-based, bladder-shaped scaffolds seeded with autologous urothelial cells and SMCs is the best option for bladder tissue engineering. Further studies to develop novel biomaterials and identify cell sources, as well as the information gained from research in developmental biology, signal transduction, and wound healing, would be beneficial.

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