8

BLADDER TISSUE ENGINEERING

Learning Objectives:

After completing this chapter, students should be able to:

1. Describe the structure, function, vascularization, and innervation of the urinary bladder.
2. Describe neurogenic bladder dysfunction and discuss complications associated with this condition and potential treatment modalities.
3. Describe surgical augmentation of the urinary bladder for patients with neurogenic bladder dysfunction.
4. Explain the development of the urinary bladder during embryogenesis.
5. List design considerations for bladder tissue engineering.
6. Describe the process of bioengineering artificial bladders using tissue engineering technology.
7. Discuss the use of cell sheet engineering for bladder tissue engineering.
8. Discuss the use of small intestine submucosa for bladder tissue engineering.
9. Discuss the use of poly(lactic-co-glycolic acid) (PLGA) for bladder tissue engineering.
10. Discuss the use of acellular scaffolds for bladder tissue engineering.
11. Describe organ models for bladder tissue engineering.
12. Describe the clinical use of artificial bladders.

Introduction to Tissue Engineering: Applications and Challenges, First Edition. Ravi Birla.

CHAPTER OVERVIEW

We begin this chapter by studying the structure and function of the urinary bladder and then look at one specific pathological condition: neurogenic bladder dysfunction. We then look at bladder augmentation as a surgical treatment for neurogenic bladder dysfunction. We also provide a brief introduction into the development of the urinary bladder during embryogenesis. We then proceed to identify the design considerations for bladder tissue engineering, and provide a general scheme for the fabrication of artificial bladders. In the rest of the chapter, we look at specific models that have been developed to bioengineer artificial bladder tissue or entire bladders. In particular, we look at cell sheet engineering to fabricate artificial bladder tissue. We also look at small intestine submucosa and PLGA as biomaterials for bladder tissue engineering. We discuss the role of acellullar grafts to support the fabrication of artificial bladders or bladder tissue. In the next section of this chapter, we look at organ models for urinary bladders. We end by discussing a seminal publication in the field that describes the use of bioengineered artificial bladders in patients.

8.1 BLADDER STRUCTURE AND FUNCTION

The bladder is part of the urinary system, which functions to regulate the composition of fluids, particularly water, in the body. The urinary system consists of the kidneys, ureters, the urinary bladder, and the urethra. In the urinary system, the kidneys are the major regulatory organ in which urine is produced and then transported through the ureters to the bladder, which serves as a storage vessel for the urine until it is removed from the body via the urethra. The kidneys, which do much of the heavy lifting in terms of fluid hemostasis, can be viewed as the primary functional and regulatory components of the urinary system. The ureters and urethra can be viewed as transport conduits to facilitate the movement of urine. And, the bladder is the primary storage location of urine until it exits the body. In this manner, the urinary system performs the critical function of fluid regulation. Any deficiencies in this process can lead to serious complications and adverse consequences.

As we have mentioned, the urinary bladder is an organ that stores urine until it exits the body. It is connected to the kidney by ureters and has a single output port, the urethra, which transports urine outside the body. From a structural and functional standpoint, the bladder is relatively simple, especially when compared to more complex organs like the kidneys. From a structural standpoint, the bladder can be viewed as a muscular container that acts to store and remove urine based on smooth muscle contraction; from a functional standpoint, the bladder can be viewed as storage vehicle for urine.

The urinary bladder consists of four layers, known as the mucosal layer, submucosal layer, muscular layer and finally, the adventitial layer (1–12). The luminal surface of the urinary bladder is known as the transitional epithelium and is followed by the lamina propria, which consists of connective tissue.Collectively, the transitional epithelium and the lamina propria are known as the mucosal layer. The

transitional epithelium consists of specialized cells that can change the number of cell layers based on bladder distension. When the urinary bladder is empty, the transitional epithelium consists of four to five layers of cells; this number is reduced to two to three when the bladder is distended. The next layer of the bladder is known as the submucosal layer and consists of areolar tissue; it serves to connect the mucosal layer to the muscular smooth muscle layer. The third layer in the bladder is the smooth muscle layer, simply referred to as the muscular layer. The smooth muscle cells within the muscular layers are aligned in two different configurations: they can be aligned in a longitudinal manner or can be aligned in a circular fashion. The smooth muscle cells in the muscular layer can be divided into three regions, starting with cells that are longitudinally aligned, followed by cells that are aligned in a circular manner and finally, cells that are again longitudinally aligned. The final layer of the bladder, which is farthest away from the transitional epithelium, consists of connective tissue, is known as the adventitial layer, and is the site for tissue vascularization.

Blood is supplied to the urinary bladder primarily through the superior and inferior vesical arteries (13–16). The superior vesical artery receives its blood supply from the patent part of the umbilical artery, which in turn receives its blood supply from the common iliac artery through the internal iliac artery. The inferior vesical artery primarily feeds the base of the urinary bladder. The obturator and interior gluteal arteries provide smaller branches to the urinary bladder. In the female urinary bladder, additional blood supply is derived from the vaginal and uterine arteries.

The urinary bladder receives stimulation from both sympathetic and parasympathetic fibers, with sympathetic stimulation from the sacral nerves and parasympathetic stimulation from the pelvic splanchnic nerves (17–19).

8.2 NEUROGENIC BLADDER DYSFUNCTION

Introduction—There are several conditions that can adversely affect bladder function, some of which include trauma, infection, cancer, loss of neurological control, and congenital disorders and abnormalities in the formation of the bladder during development. In these cases, the bladder loses the ability to perform its primary function: the storage and removal of urine from the body. In this section, we will look at one specific example of a neurological disorder of the bladder: neurogenic bladder. In cases of neurogenic bladder, a person loses control of bladder functions due to damage and/or injury to the nervous system (20–33). In addition to loss of bladder function, neurogenic bladder increases the risk of kidney failure. Neurogenic bladder can be congenital, resulting from birth defects associated with the spinal cord formation; or can be acquired due to injury of the nerves that innervate the bladder, resulting from injury or trauma. These problems can lead to an overactive bladder, which refers to an increase in the frequency of urination, or an underactive bladder, in which case the bladder does not empty even when full and can leak.

Complications Associated with Neurogenic Bladders—There are several complications that occur as a result of neurogenic bladders, including hydronephrosis, renal failure, urinary tract infections, calculus diseases, and cancer of the bladder. Hydronephrosis is a condition in which water accumulates in the kidneys due to inefficient removal from the body resulting from a poorly functioning bladder. This condition is a very severe and serious consequence of neurogenic bladder; it can lead to loss of renal function and eventually lead to patient mortality. In addition to hydronephrosis, renal failure can also occur due to pyelonenephritis, which is a urinary tract infection affecting the kidney; it can lead to kidney failure and can prove to be fatal. Urinary tract infections are associated with the accumulation of urine and the inability of the bladder to remove the accumulated urine from the body. Another complication steming from neurogenic bladders is calculus disease, which refers to the formation of stones in the urinary tract due to the accumulation and/or immobility of the urine. The risk of bladder cancer is also significantly higher in cases of neurogenic bladders due to infections and bladder stones.

Catheterization Management of Symptoms Relating to Neurogenic Bladders—Catheterization is the standard procedure for the management of neurogenic bladder dysfunction. In this procedure, a catheter is inserted directly in the urinary bladder by passing it through the urethra. The catheter can be used to drain urine from the bladder by way of gravity flow. Catheterization can be for short durations, in which case it is referred to as intermittent catheterization (IC), or can be long-term, in which case the catheter is retained for 30 days or longer. In the case of IC, the patient is typically able to insert the catheter and can do so on a regular basis, typically 4–6 times per day. This process is designed to simulate normal urinary function. In the case of long-term catheterization, the catheter is retained for an extended period of time. The Foley catheter, manufactured by Barb Medical, is an example of a commercially available catheter used for extended time periods. While long-term catheterization offers the advantage of continuous draining, it also significantly increases the risk of urinary tract infection due to the direct route from the external environment to the urinary tract.

Pharmacological Treatment of Neurogenic Bladders—Anticholinergic drugs are effective in the management of overactive bladders. They act by relaxing by the detrusor muscle in the bladder wall. Examples of anticholinergic drugs that are commonly used include oxybutynin, trospium chloride, and propiverine. Anticholinergic agents work by blocking the activity of acetylcholine, which is a neurotransmitter that activates the detrusor muscle in the bladder wall leading to muscle contraction and drainage of urine form the bladder. Inactivation of acetylcholine will prevent stimulation and, therefore, contraction of the detrusor muscle and will act to reduce the frequency of contraction in overactive bladders.

Neuromodulation as a Therapeutic Strategy for Neurogenic Bladders—The term neuromodulation refers to electrical stimulation of the nerves that innervate the bladder. As we have seen before, neurogenic bladder is a condition that occurs due to the loss of nerve stimulation. Therefore, it has been hypothesized that utilization of electrical stimulation can regulate bladder function in a way that is similar to the nerves that innervate it. In other words, neuromodulation provides

a mechanism to restore lost nerve innervation to the bladder (34–39). Electrical stimulation has been used extensively as a therapeutic strategy for many medical conditions and has been discussed in Chapter 6. In addition, electrical stimulation has been shown to correlate with the development and maturation of bioengineered artificial tissue, including artificial heart muscle. this material was presented in Chapter 6. Here, we discuss another application of electrical stimulation: as a therapeutic modality for the treatment of neurogenic bladders. In two separate studies, electrical stimulation of the sacral nerve has been conducted as a treatment for neurogenic bladder dysfunction in adult and pediatric patients (40,41). In the study with adult patients, neuromodulation was accomplished by electrodes that were inserted into one or both dorsal foramina of the S3 segment (41). While there were variations in the results of this study, the symptoms were reduced by at least 50% in 30% of the patients (41). In the second study, sacral nerve stimulation was evaluated in pediatric patients; as in the case of adult patients, there were variations in the results, although the general trend was toward functional improvement in a large percentage of the patients (40).

8.3 SURGICAL BLADDER AUGMENTATION

A surgical procedure known as bladder augmentation is an option for patients with neurogenic bladder disorders or other abnormalities of the lower urinary tract. Bladder augmentation refers to the process by which the urinary bladder is expanded by suturing autologous segments of the ileum, small or large bowel, or segments of the ureters (42–53). Surgical intervention is only considered when other treatment strategies have failed to provide the desired functional benefit. Patients with neurogenic bladder disorders will first be treated with pharmacological agents like anticholinergic compounds, while urinary incontinence is managed by use of catheters. Surgical intervention for bladder augmentation can be considered when these procedures fail to provide significant functional benefits to the patient. Once the decision to proceed with bladder augmentation has been made, one of the most important decisions is regarding the choice of tissue specimen to be used. Some of the choices include gastric segments, small and large bowel segments, segments of the ureter, and segments of the ileum. The use of ileac segments for bladder reconstruction is known as ileocystoplasty and will be described here.

The primary objective of bladder augmentation is to increase the volume of the bladder and increase total muscle mass by suturing autologous tissue specimens, as muscular tissue is used as the graft. An increase in bladder pressure is known to adversely affect renal tissue function, and by decreasing this pressure, renal function can be preserved. The increase in muscle mass serves to increase the ability of the bladder to remove urine to the external environment. Therefore, bladder augmentation surgery can increase the functional performance of the urinary bladder by reducing internal pressure, preserving renal function, and increasing muscle mass and contractile activity.

As we have mentioned before, ileocystoplasty is the surgical augmentation of the bladder using a segment of the ileum. An ileac segment with a length of about 30 cm is excised from the patient, and the remaining portions of the ileum are reconnected. The excised portion of the ileum is flushed and opened by making an incision at the midline, and a pouch is created by anastomosis of the opposite ends of the excised ileum. This process leads to the fabrication of an autologous tissue graft, which can then be used for bladder augmentation. An incision is made in the bladder, the tissue graft is sutured on the open bladder, and the open bladder with the sutured ileum segment is closed by anastomosis of the open ends. This process expands the bladder by increasing its internal volume and increases the muscular component of the bladder; it preserves renal function and supports urine discharge from the bladder.

8.4 DEVELOPMENT OF THE URINARY BLADDER

During embryogenesis, the cloaca, Wolffian ducts, and the caudal nephric duct are important structures that give rise to the urinary bladder (54–56). The cloaca is a sac that contains endodermal cells. The lower region of the sac is referred to as the urogenital sinus, which contains the cells which give rise to the urinary bladder and the urethra. The location of the urogenital sinus during embryonic development is aligned with the location of the fully formed bladder and urethra. The Wolffian ducts (WDs), also known as the mesonephric ducts, and the nephric duct consist of two tubes connecting the primordial kidney to the cloaca, eventually leading to the formation of the trigone region of the urinary bladder. During one of the earlier steps of embryogenesis, the Wolffian duct inserts within the cloaca, and later on, the ureter develops and migrates from the primordial kidney toward the cloaca. As the ureter approaches the cloaca, it forms a common tubular connection with the Wolffian duct, and the region of shared connection is known as the common nephric duct (CND), also referred to as the caudal nephric duct. During the course of development, the cells of the CND undergo apoptosis, allowing the ureter to form an insertion with the cloaca, which is independent of the Wolffian duct. Using this mechanism, the two ureters form and are inserted within the clocaca; subsequent maturation of the cloaca leads to formation of the bladder and the urethra.

8.5 DESIGN CONSIDERATIONS FOR BLADDER TISSUE ENGINEERING

In the previous chapter, we looked at the design considerations for tracheal tissue engineering; many of the design considerations that were presented for tracheal tissue engineering also apply for the fabrication of artificial bladders. In the previous chapter, we also presented an overarching design statement for tracheal tissue engineering, which also applies for bladder tissue engineering: *bioengineered bladders should be similar in form and function to mammalian bladders*.

The specific design considerations for bladder tissue engineering are: 1) bioengineered bladders must serve several important functions, including the storage of urine and the timely and efficient removal of the urine from the body, 2) biocompatibility, 3) nonimmunogenic and minimal inflammatory response, 4) nontoxic and noncarcinogenic, 5) avoidance of collapse by reasonable strength, 6) support cell engraftment, 7) support neovascularization, 8) possibility of growth, 9) resistance to fibroblastic and bacterial invasion, 10) standardized easy and short fabrication, 11) customizable and low cost, 12) easy surgical handling, 13) provide physiological environment such as ECM, 14) minimal necessity of donors and accessibility, 15) the results of engraftment are predictably successful, 16) provide or support epithelial resurfacing, 17) must not dislocate or erode over time, and 18) durability.

8.6 PROCESS OF BIOENGINEERING ARTIFICIAL BLADDERS

Introduction—In Chapter 1, we presented a general scheme to bioengineer artificial tissue, and in Chapter 7 we presented a scheme to fabricate artificial tracheas. There were similarities between the two processes, although there were significant differences, particularly related to the tissue fabrication and cellularization strategies. In this section, we present a general process flow sheet for the fabrication of artificial bladders, along with a discussion of cell sourcing, biomaterials, and tissue fabrication technology (Figure 8.1). The process flow sheet is generic and does not represent any specific technology from any research laboratory. In subsequent sections, we will present specific examples of strategies that have been used to bioengineer artificial bladders.

Tissue Graft Development—There are three tissue engineering strategies that can be used for the fabrication of artificial bladder tissue and/or entire bladders: 1) bioengineer an entire artificial bladder, 2) bioengineer a patch or graft that can be sutured with the host graft, similar to tissue grafts used in bladder augmentation surgery and 3) bioengineer partial bladders that can be sutured onto host tissue to support and/or improve bladder function. These three bioengineering strategies are presented in Figure 8.1. In the first case, an entire bioartificial bladder can be fabricated in the laboratory and used for organ transplantation. This strategy involves fabrication of an entire organ and therefore is associated with significant scientific and technology challenges that must first be overcome. The second strategy is designed to fabricate tissue patches that can be used in bladder augmentation. During our discussion of bladder augmentation, we saw that tissue grafts are often derived from gastric segments, segments of small and large bowel, segments of the ureter, and segments of the ileum. This requires incision of tissue grafts from the host followed by reanastomosis with host bladder tissue. This is a very invasive surgery that can be prevented by the use of bioengineered tissue grafts, which can be directly sutured with the host bladder. The third strategy is based on the ability to bioengineer partial bladders. While this is similar to the development of tissue grafts, it is focused on the fabrication of hollow chambers rather than planar scaffolds. This strategy is based on the fabrication of segments of hollow chambers

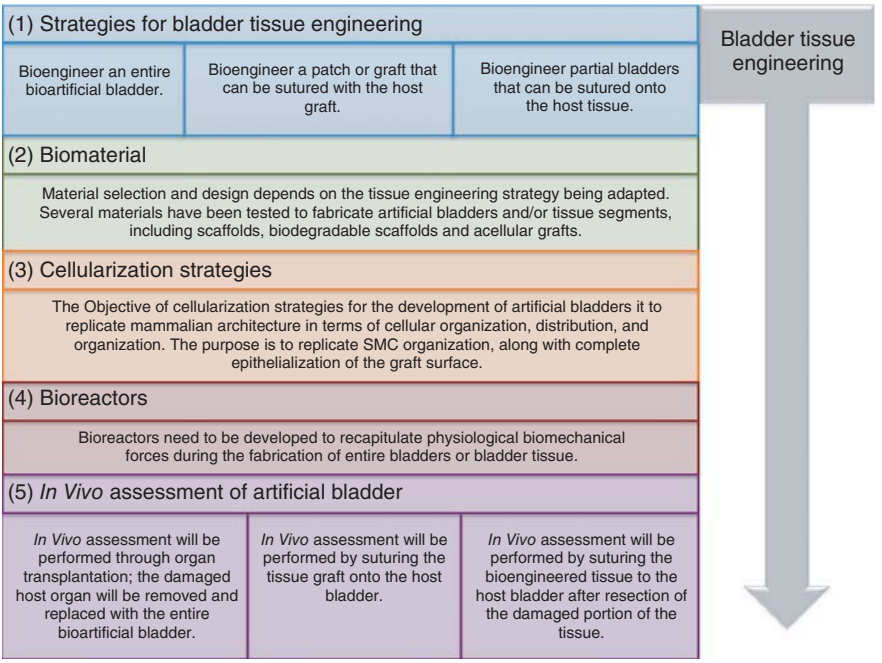

Figure 8.1 Process of Bioengineering Artificial Bladders—Three strategies for bladder tissue engineering are presented: fabrication of entire bladders, partial bladders, or bladder tissue segments. Many different biomaterial platforms have been used, including acellular grafts, biodegradable hydrogels, and polymeric scaffolds. Cellularization strategies have been focused on epithelization of the luminal surface with smooth muscle cells within the interior. Bioreactors need to be developed to simulate natural bladder function. Finally, several models have been developed to test the effectiveness of artificial bladders and/or artificial bladder tissue *in vivo*.

which can be sutured with the host bladder; compared to planar tissue grafts, hollow structures can provide greater functionality.

Biomaterial Design and Fabrication—Several materials have been tested to fabricate artificial bladders and/or tissue segments, including synthetic scaffolds, biodegradable scaffolds, and acellular grafts, with varying degrees of success. Material selection and design depends on the tissue engineering strategy being adapted, with greater mechanical strength and stability required when fabricating hollow grafts and entire bioartificial bladders than when performing planar tissue grafts. As was the case for tracheal tissue engineering, acellular scaffolds have been proven to be successful in artificial bladders that have been used clinically in patients.

Cellularization for the Fabrication of Artificial Bladders—The objective of cellularization strategies for the development of artificial bladders is to replicate mammalian architecture in terms of cellular organization, distribution, and organization.

As we have seen earlier in this chapter, the urinary bladder is composed of epithelial cells on the outer surface and smooth muscle cells within the interior. In addition, the smooth muscle cells (SMCs) were organized in three distinct layers consisting of longitudinal cells, circular cells, and longitudinal cells. Tissue engineering strategies are focused on replicating this complex organization of SMCs, along with complete epithelialization of the graft surface.

Bioreactors—Interestingly, bioreactors have not played a prominent role in supporting the fabrication and/or maturation of artificial bladder tissue. While the bladder is not exposed to extensive hemodynamic forces like the heart or blood vessels are exposed to, it is exposed to external forces on a frequent basis. For example, the bladder is constantly exposed to changes in the volume of urine, which is accompanied by changes in the stress fields on the walls of the urinary bladder. Similarly, removal of urine from the bladder is due to smooth muscle contraction, which results in significant changes in the pressure-volume relationship. These biomechanical forces have an impact on cell phenotype and tissue function, as the effects of stress on endothelial cells and smooth muscle cells are well documented. Therefore, there is a need to develop bioreactors to recapitulate physiological biomechanical forces during the fabrication of entire bladders or bladder tissue. However, bioreactor design and fabrication has not been a high-priority area of research; there are very few publications in this area, as we will see in subsequent sections.

In Vivo Functional Assessment of Artificial Bladders or Tissue Equivalents—The final step in the process scheme is the development of *in vivo* models to assess the functionality of bioengineered tissue grafts and/or entire artificial bladders. In the first case, which involves the fabrication of an entire bladder, *in vivo* assessment will be performed through organ transplantation. The damaged or diseased host organ will be removed and replaced with the bioartificial bladder, ensuring connectivity to the ureters and the urethra. In the second case, which involves the fabrication of planar grafts, *in vivo* assessment will be similar to what has been described for bladder augmentation surgery, and the tissue graft will be sutured onto the host bladder. In this case, an incision will be made to open the bladder, and the bioengineered graft will be sutured onto the host bladder. In the third case, which involves the fabrication of partial bladder components, the bioengineered tissue can be sutured to the host bladder after resection of the damaged or diseased portion of the tissue.

8.7 CELL SHEET ENGINEERING FOR BLADDER TISSUE ENGINEERING

Introduction—During our discussion of tissue fabrication technologies in Chapter 4, we looked at cell sheet engineering. As a reminder, cell sheet engineering is a scaffold-free technology, which relies on ECM produced by the cells to support 3D tissue formation and function. Isolated cells are plated on a temperature-sensitive culture surface, and cell culture conditions are optimized to support the formation of a cohesive cell monolayer; the temperature of the

tissue culture plate is then reduced, resulting in detachment of the cohesive cell monolayer. Individual cell sheets are fabricated using these temperature-sensitive surfaces, and individual sheets can be stacked together to form thicker multilayer tissue. The primary advantage of using cell sheet engineering to fabricate artificial tissue is that ECM is produced by the cells and contains the right composition of proteins and other ECM components to support 3D tissue formation. ECM that is fabricated by the cells is very well-suited to support cell attachment, viability, and functionality; the ECM fabricated by the cells leads to the fabrication of functional 3D artificial tissue. Cell sheet engineering has been used for the fabrication of artificial bladders, as will be presented in the remainder of this section (57,58).

Cell Sourcing—This study was conducted using epithelial cells that were isolated from bladder tissue of dogs (57). However, the study did not take into account the smooth muscle cell layer of the bladder tissue, and did not incorporate a muscular layer in the artificial bladder. The primary epithelial cells were isolated from dog bladder, which was cut into small pieces, and the epithelial layer was separated from the underlying tissue. This epithelial tissue was suspended in an enzymatic solution to separate the cells from the tissue.

Biomaterials—Cell sheet engineering technology is based on scaffold-free methods, which rely on the ECM generated by the cells. The cells fabricate ECM and then use the newly formed ECM to fabricate 3D artificial tissue. Tissue fabrication does not require any external scaffolding.

Tissue Fabrication Technology—Primary cells were cultured on temperature-sensitive surfaces for a period of three weeks. During the culture period, the tissue culture plates were maintained in a cell culture incubator at 37°C, with frequent media changes. A three week culture period was necessary to support the formation of a cohesive cell monolayer and to promote ECM production by the cells. At the end of this culture period, the temperature was reduced from 37°C to 20°C, which resulted in detachment of the cohesive cell monolayer from the underlying surface. After detachment, the cells had formed a cohesive cell monolayer that can be referred to as a cell sheet.

In Vivo Testing—In order to test the cell sheets during *in vivo* implantation, a novel strategy was developed for autologous cell transplantation (58). However, in order to support tissue fabrication and implantation of artificial tissue, autologous cells were not used, as complications can result from using a single bladder to harvest primary cells and for implantation of artificial tissue. In order to overcome this problem, primary cells were isolated from the oral cavity and used to bioengineer cell sheets using the technology described for epithelial cells isolated from bladder tissue (58). The cell sheets were used in conjunction with a gastric flap as a graft for bladder reconstruction, as the cell sheets alone do not have the mechanical strength to support bladder function. The cell sheets, fabricated with epithelial cells from the oral cavity, were placed on top of the gastric flap and used for bladder construction. A section of the host bladder was resected and replaced with the gastric flap with the cell sheet attached to it.

Discussion of Cell Sheet Engineering for Bladder Tissue Engineering—The use of cell sheet engineering for bladder reconstruction was used in conjunction with

bladder augmentation. Gastric flaps were coupled with cell sheets and then used for transplantation. The primary functional component was provided by the gastric flap, while the cell sheets served to provide a supporting role. This is somewhat expected, as cell sheets do not possess the mechanical properties to support the fabrication of complex hollow organs like the bladder. Significant advancement of the cell sheet technology will be required prior to development of complex organs like the urinary bladder. However, cell sheet engineering remains a novel technology that has been used extensively for many tissue fabrication applications.

8.8 SMALL INTESTINAL SUBMUCOSA (SIS) FOR BLADDER TISSUE ENGINEERING

Introduction—Small intestinal submucosa (SIS) has been extensively used for bladder tissue engineering (Figure 8.2).

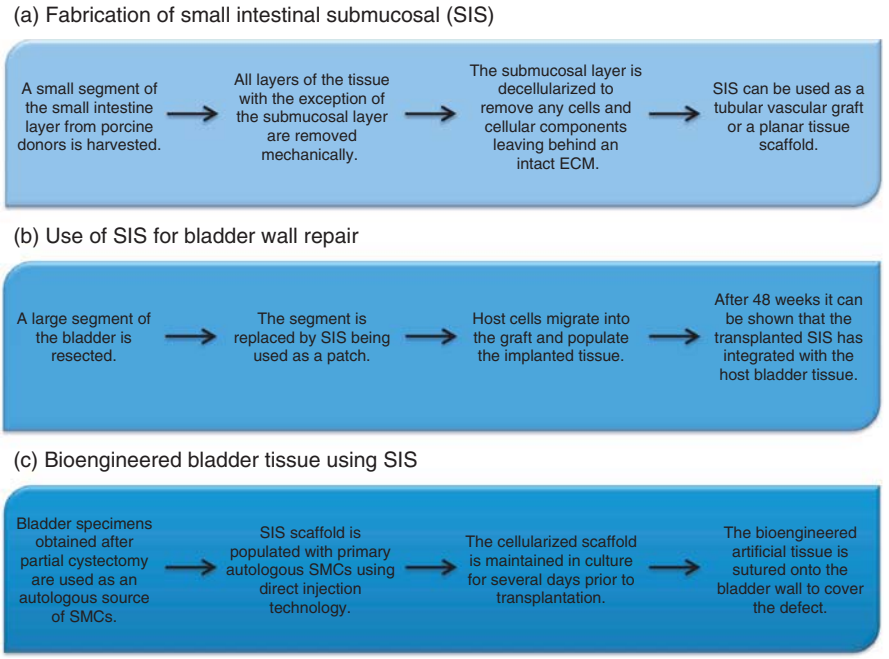

Figure 8.2 SIS for Bladder Tissue Engineering—(a) Fabrication of SIS—SIS is fabricated from a segment of the small intestine after removal of all layers of the tissue except the submucosal layer. **(b) Use of SIS for Bladder Wall Repair**—SIS is used, in the absence of cells, for bladder reconstruction. **(c) Bioengineered Bladder Tissue using SIS**—SIS is populated with cells using direct cell injection and the artificial tissue graft is used for bladder augmentation.

SIS is a very interesting biomaterial that has been used for many different tissue engineering applications (59–68), and one that we have not discussed before. We begin this section with an overview of SIS as a biomaterial and then provide specific examples of the use of SIS for bladder reconstruction and bladder tissue engineering. We will look at two examples, one of which describes the utilization of SIS as a biomaterial (in the absence of cells) for bladder repair and the second example, in which artificial bladder tissue is fabricated by cellularization of the SIS biomaterial, which is then used as a graft for bladder tissue repair.

Small Intestinal Submucosa as a Biomaterial—SIS is obtained from the submucosal layer of a small intestine segment that has been harvested from porcine donors (64). The small intestine consists of several distinct tissue layers with an epithelial layer on the luminal surface, followed by goblet cells, the submucosal layer, and longitudinally and circularly aligned muscle cells. During the preparation of SIS, a segment of the small intestine layer is harvested, commonly from pigs, and all layers of the tissue, with the exception of the submucosal layer, are removed mechanically. The submucosal layer is next subjected to a decellularization protocol to remove any cells and cellular components, leaving behind an intact ECM. This two-step process that consists of mechanically removing layers of porcine small intestine followed by decellularization results in the production of SIS. SIS can be used as a tubular vascular graft or can be transformed to form a planar tissue scaffold by making an incision along the length of the tubular graft. Since SIS is biologically derived, it contains several ECM components, including collagen, proteoglycans, GAGs, glycoproteins, and growth factors; these components make SIS an attractive biomaterial for tissue engineering applications to support cell attachment, remodeling, and subsequently, tissue fabrication. The primary advantage of SIS is the biologically derived ECM, which contains proteins and other components of the ECM that support cell attachment and spreading and support cell-matrix interactions; these functions in turn modulate cell phenotype and support 3D tissue fabrication.

SIS as a Xenogeneic Material—It should be noted that SIS is a xenogeneic material, which means that it is isolated from animal donors, and after being processed using specific conditions, it can be designed and used for transplantation. In the case of xenogeneic transplantation, cells, tissue, or entire organs obtained from one species, commonly pigs, are used for transplantation in a different species, typically humans. This can be compared to autologous and allogeneic sources, which were discussed in Chapter 2 in the context of cell sourcing. In the case of autologous transplantation, the donor and recipient of cells or biomaterials are the same, while in the case of allogeneic transplantation, the donor and recipient are different but from the same species. The primary advantage of autologous transplantation is immune acceptance by the recipient. Immune rejection is a concern with allogeneic and xenogeneic transplantation. However, SIS is nonimmunogenic; cell surface antigens are responsible for host immunogenic response, and xenogeneic materials like SIS are nonimmunogenic due to the complete removal of the cellular components.

SIS as a Bladder Wall Substitute—There have been several studies demonstrating the utility of SIS for bladder reconstruction using tissue engineering as well as

alternative strategies (69,70). In one study conducted on rats, SIS was used as a patch for bladder augmentation surgery (70). A large segment of the bladder was resected and replaced with SIS, which was sutured onto the remaining bladder wall (70). After a period of 48 weeks, it was shown that the transplanted SIS had integrated with the host bladder tissue, and the two were indistinguishable (70). In addition, the implanted graft had formed three layers of bladder tissue: the epithelium, the smooth muscle layer, and the serosa. The SIS was not cellularized at the time of implantation, but host cells migrated into the graft and populated the implanted tissue, supporting the formation of bladder tissue.

This was a very simple application of SIS biomaterial for bladder wall substitution. Since there were no cells involved in the study, this strategy is not considered to be tissue engineering. However, there are several examples in the recent literature where biomaterials (without cells) have been used for the repair of damaged and/or injured tissue. This study clearly demonstrated the integration of SIS with bladder tissue, and the results of this study suggest that SIS can be used as a tissue graft for bladder augmentation surgery. In addition, the results of this study suggest that SIS may have the potential to be bioengineered into artificial bladders, and the coupling of the biomaterial with cells may provide additional functional benefit. The ability of SIS to functionally integrate with host tissue over a period of 48 weeks and withstand the constant changes in the bladder pressure clearly demonstrates the potential of the biomaterial in bladder reconstruction and bladder tissue engineering.

Cell-Seeded SIS Graft for Bladder Reconstruction—In the previous example, we looked at the use of SIS as a biomaterial for bladder reconstruction without the incorporation of cells. However, the utility of SIS to support engraftment of cells and then use the cell-seeded graft for bladder reconstruction has also been evaluated. In a recent study, SIS grafts were fabricated from small intestine tissue after mechanical removal of all tissue layers except the submucosal layer followed by decellularization of the submucosal layer to remove any remaining cells (69). New Zealand white rabbits were used for bladder augmentation studies and were subjected to a partial cystectomy to create an injury model. The tissue specimens that were obtained after the partial cystectomy were used to isolate SMCs, which were cultured and expanded on tissue culture plates. After partial cystectomy, the animals were housed for one month and used as recipients for tissue engineered grafts. Using this strategy, the researchers were able to use an autologous cell source to bioengineer artificial bladder tissue; SMCs were isolated from New Zealand White rabbits and used to bioengineer tissue grafts, which were later implanted into the same animal. In order to support the formation of artificial bladder tissue, the SIS scaffold was populated with primary autologous SMCs using direct injection technology. The cellularized scaffolds were maintained in culture for several days prior to transplantation and then used as a tissue graft for bladder augmentation. The bioengineered artificial tissue was sutured onto the bladder wall to cover the defect, and the host bladder was evaluated at several time intervals after transplantation of the tissue graft.

Discussion—In this section, we have seen two contrasting strategies for bladder tissue repair; one relies upon the use of a biomaterial for bladder tissue augmentation, while the second one uses bioengineered artificial tissue for bladder repair. Stated another way, the first strategy is based on the use of biomaterials for tissue repair, while the second strategy is based on biomaterials and cells for tissue repair. When a biomaterial is used without cells, the strategy is based on the hypothesis that host cells will migrate into the implanted biomaterial to support the formation of functional tissue. While both strategies continue to be aggressively researched and developed, we believe that the latter of the two offers greater benefit due to the presence of cells at the time of graft implantation.

We will end our discussion in this section by relating some of the principles of tissue engineering presented in earlier chapters with the models that have been discussed here. It should be noted that in the tissue engineering study (the second of the two presented), primary cells were used and were isolated from an autologous source. The cells were maintained and expanded in culture using standard cell culture procedures. The SIS biomaterial used in the study was obtained using a deceullarization strategy and populated with cells using direct injection technology. Many of the terms used here (primary cells, autologous, decellularization, cell culture, and direct cell injection) have been discussed in earlier chapters, and the reader is encouraged to relate these fundamental principles of tissue engineering to the specific examples of bladder tissue engineering.

8.9 PLGA AS A BIOMATERIAL FOR BLADDER TISSUE ENGINEERING

Introduction—In the previous section, we looked at SIS as a biomaterial for bladder augmentation both with and without cells. In this section, we will study another such example. In this section, we will study the use of the polymeric scaffold poly (lactic-co-glycolic acid), PLGA, to support bladder repair, and as in the previous example, we will look at the use of PLGA with and without cells (Figure 8.3). PLGA has been used extensively as a biomaterial for tissue engineering along with many other medical applications (71–79). We begin this section with a discussion of the properties of PLGA and then move onto our discussion of the use of this material for bladder repair and augmentation.

PLGA as a Biomaterial—PLGA has been extensively used as a vehicle for controlled delivery of therapeutic agents and as a porous scaffold for tissue engineering applications. PLGA is a degradable copolymer of lactic acid and glycolic acid; it is often described in terms of the relative percentage of these two monomers; PLGA 25:75 refers to PLGA with a composition of 25% lactic acid and 75% glycolic acid (79). One of the main advantages of PLGA is the nontoxicity of its degradation products; PLGA undergoes hydrolysis, and the degradation products of this reaction are the monomers lactic acid and glycolic acid, both of which are easily metabolized by the body (79). The degradation kinetics of PLGA can be modulated over a period of weeks or months by changing the relative proportions of monomer units. The ability to control the degradation kinetics of PLGA has made

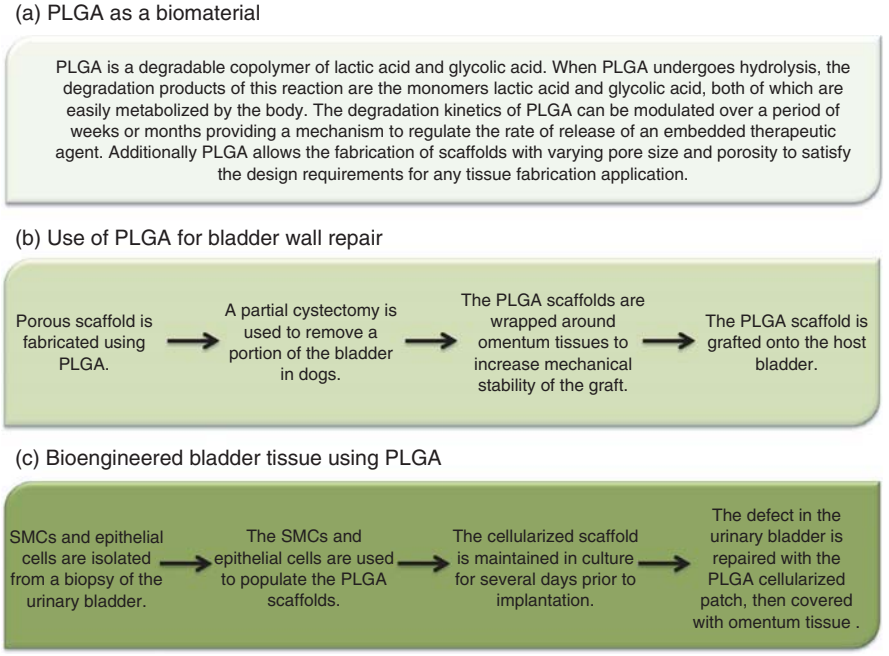

Figure 8.3 PLGA for Bladder Tissue Engineering—(a) PLGA as a Biomaterial– PLGA is a copolymer of lactic acid and glycolic acid. **(b) Use of PLGA for Bladder Wall Repair**—3D scaffolds were fabricated using PLGA and used for bladder augmentation without the use of any cells in the scaffold. **(c) Bioengineered Bladder Tissue Using PLGA**—3D scaffolds were populated with cells prior to use for bladder augmentation.

it a suitable biomaterial for controlled release strategies. Regulating the degradation kinetics of PLGA provides a mechanism to regulate the rate of release of an embedded therapeutic agent.

PLGA has also been widely used as a porous scaffold for various tissue engineering applications (79–96). There have been several techniques described for the fabrication of PLGA into porous scaffolds, and the properties of the scaffolds, like the pore size and porosity, can be modulated by varying the fabrication process; this modulation provides the ability to fine-tune the properties of the porous scaffold to satisfy the design requirements for any tissue fabrication application.

PLGA Scaffolds for Bladder Reconstruction—In one particular study, porous scaffolds were fabricated using PLGA, though the details of the fabrication technology were not presented in the published manuscript. The PLGA scaffolds were implanted in dogs after a partial cystectomy to remove a portion of the host bladder (97). The PLGA scaffolds were not used alone, but rather wrapped around omentum tissue at the time of implantation in order to increase the mechanical stability of the graft. Bladder function was assessed at various time intervals after implantation of the tissue grafts. Functional performance of the bladder was assessed by measuring

the bladder capacity and bladder compliance, both of which showed improvement after implantation of the PLGA scaffold.

Cellularized PLGA Scaffolds for Bladder Reconstruction—PLGA scaffolds were cellularized using autologous SMCs and epithelial cells that were isolated from a biopsy of the urinary bladder (98). The cells were maintained in culture and subpassaged to increase the number of cells. SMCs and epithelial cells were used to populate the PLGA scaffolds. The details of the cellularization protocol were not provided in the study, but it can be assumed that direct injection technology was used for scaffold cellularization. The cellularized scaffolds were maintained in culture for several days prior to implantation. The implantation model for the cellularized scaffolds was similar to that for the PLGA scaffolds without cells. A partial cystectomy was performed to remove part of the urinary bladder in dogs, repaired with a cellularized patch, then covered with omentum tissue. As before, biological and functional performance metrics were evaluated. It was found that the cellularized PLGA patch performed better than the PLGA patch without cells both in terms of functional performance and in terms of histological data.

Discussion of the Model—In this example, we have seen the use of PLGA, a biodegradable biomaterial, to support the functional repair of bladder tissue. The PLGA was used with and without cells in a side-by-side comparison, and the cellularized scaffold performed better than the scaffold without cells. This study serves to demonstrate the clear advantages of tissue engineering strategies over other methods and showcase the ability of cell-seeded scaffold to support functional recovery of bladder tissue. In addition, the study also illustrates many important principles of tissue engineering that we have discussed in earlier chapters: autologous cells, cell culture, biodegradable scaffold, and direct injection technology.

8.10 ACELLULAR GRAFTS FOR BLADDER TISSUE ENGINEERING

Introduction—In this section, we will look at the use of acellular grafts for bladder tissue engineering; there have been several examples in the literature demonstrating the feasibility of acellular grafts to support the formation of artificial bladder tissue (Figure 8.4). We provide three specific examples in this section: 1) use of acellular grafts without the incorporation of cells, 2) use of acellular grafts with the incorporation of cells, and 3) the use of acellular grafts with the incorporation of adipose-derived stem cells.

Acellular Grafts for Bladder Tissue Engineering—We have been introduced to acellular grafts as a scaffold for tissue engineering applications during our discussion of biomaterials. Acellular scaffolds are fabricated by complete removal of cellular components from tissue/organ specimens that have been harvested from animal or human sources. After removal of all the cellular components, an intact ECM is retained. The retained ECM is rich is ECM proteins and other components of the ECM. This acellular graft has binding sites for cells and supports cell-matrix interactions, leading to the formation of functional artificial tissue. These properties

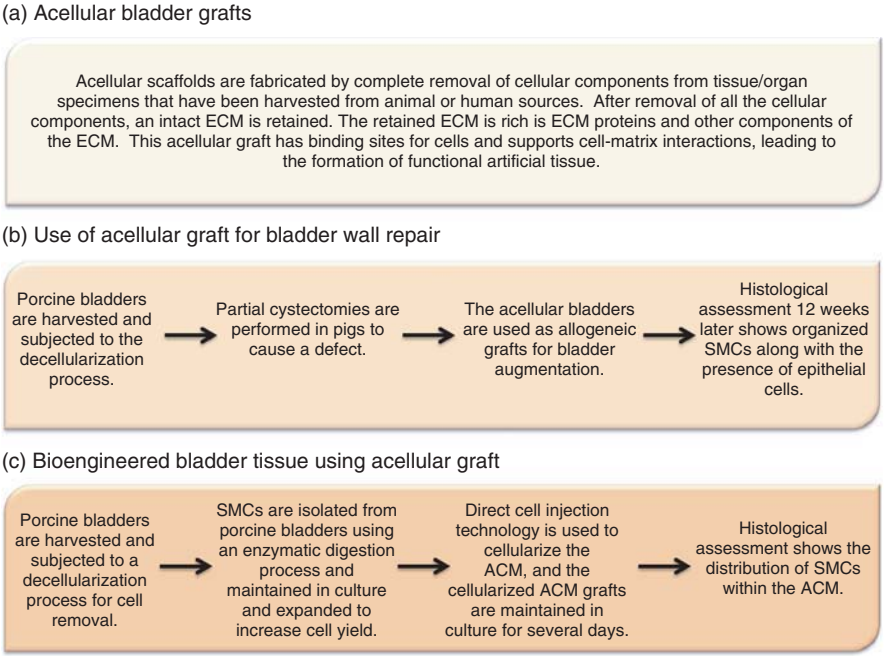

Figure 8.4 Acellular Scaffolds for Bladder Tissue Engineering—(a) Acellular Bladder Grafts—Acellular grafts are fabricated by the complete removal of cells, leaving behind an intact extracellular matrix. **(b) Use of Acellular Grafts for Bladder Wall Repair**—In this case, acellular grafts were used in the absence of any cells. **(c) Bioengineered Bladder Tissue using Acellular Grafts**—Acellular grafts were populated with cells and then used for bladder augmentation.

of acellular grafts have proven to be important in the fabrication of artificial tissue for many different tissue and organ systems, including the urinary bladder, as we will see in this section.

Use of Bladder Acellular Matrix Allograft (BAMA) for Bladder Augmentation—In this study, porcine bladders were harvested and subjected to a detergent- and enzyme-based decellularization process to remove all the cells (99,100). Partial cystectomies were conducted in pigs, and the acellular bladders were used as allogeneic grafts for bladder augmentation. During an initial short-term study, animals were sacrificed after 30 days; histological assessment showed that scattered SMCs had infiltrated the acellular bladder grafts. In a follow-up study, animals were sacrificed after 12 weeks, and histological assessment showed organized SMCs along with the presence of epithelial cells. Combined, these two studies demonstrated the feasibility of using acellullar bladder grafts to support bladder repair (99,100). It should be noted that the acellular grafts were not populated with cells at the time of transplantation; rather, this strategy relies upon infiltration of host cells to populate the transplanted graft. This is similar to the strategy that was presented for SIS earlier in this chapter.

Cell-Seeded Acellular Matrix (ACM) for Bladder Reconstruction—In a recent study, porcine bladders were harvested and subjected to a decellularization process for cell removal (101). SMCs were isolated from porcine bladders using an enzymatic digestion process. The SMCs were maintained in culture and expanded to increase cell yield. Allogeneic SMCs were used to populate the ACM; this study was designed for *in vitro* testing and not for *in vivo* bladder augmentation. These studies were designed for model development and assessment. Therefore, the use of allogeneic cells was completely justifiable. Once a sufficient number of cells were obtained, the cells were used to populate the ACM. Direct cell injection technology was used to cellularize the ACM, and the cellularized ACM grafts were maintained in culture for several days. Histological evidence showed the distribution of SMCs within the ACM and provided early evidence for the formation of artificial bladder tissue.

Use of Adipose-Derived Stem Cells for Bladder Tissue Engineering—A very interesting study was published that makes use of adipose-derived stem cells to populate acellular bladder grafts and uses cellularized artificial tissue for bladder repair (102). In this study, bladders were harvested from New Zealand White rabbits and subjected to a decellularization protocol for cell removal. A tissue biopsy was obtained from the backs of the rabbits and subjected to a collagenase digestion protocol for the isolation of adipose-derived stem cells. These cells were maintained and expanded in culture and used to populate segments of an acellular bladder; autologous cells were used for this study. The cellularized tissue was maintained in culture for several days and then used as a graft for bladder reconstruction. A defect was created in the bladder wall, and the bioengineered bladder tissue was sutured on the site of the defect. The grafts were implanted for a period of 24 weeks, after which the animals showed improvement in bladder function when compared to controls (acellular grafts without any cells). Perhaps more interesting was the differentiation of adipose-derived stem cells to form SMCs and epithelial cells, as demonstrated by positive staining for cell specific markers; the differentiation of the stem cells was likely mediated by cues within the *in vivo* environment.

Discussion of Acellular Grafts for Bladder Tissue Engineering—Acellular grafts have proven to be successful for bladder augmentation either alone or in combination with cells. When acellular grafts were used in the absence of cells, infiltration of cells from the host tissue leads to cellularization of the acellular grafts. However, this process is difficult to regulate, and the spatial distribution of the cells cannot be modulated. This limitation can be overcome by the use of cell-seeded scaffolds in which the distribution and organization of the cells can be controlled. In the three models that have been discussed, we have seen the use of allogeneic SMCs for studies designed for *in vitro* testing and have seen the use of autologous adipose-derived stem cells for *in vivo* bladder augmentation. In this section, we have seen many of the concepts that were described in earlier chapters, some of which include acellullar scaffolds, autologous and allogeneic cells, direct injection technology, and adipose-derived stem cells.

8.11 ORGAN MODELS FOR BLADDER TISSUE ENGINEERING

Introduction—We have looked at several tissue engineering models for fabricating artificial bladder tissue. However, in all of the examples, the focus has been on the development of artificial bladder tissue and not entire hollow chamber organs. The development of artificial bladder tissue is important, as it provides valuable information about the organization and the remodeling of isolated cells to form functional 3D artificial tissue. In addition, artificial bladder tissue can also be used as a patch to support and/or augment function in injured or diseased bladders. Undoubtedly, tissue models play a vital role in the development of tissue engineering therapies to support bladder function. In addition to the development of tissue models, several research groups have developed organ models that more closely replicate organ-level architecture of the urinary bladder. In this section, we will look at two examples of organ models for bladder tissue engineering, one of which was developed using a novel *in vivo* approach and one of which was developed using a novel *in vitro* approach.

In Vivo Organ Model for Bladder Tissue Engineering—A very interesting strategy was developed for the fabrication of hollow chamber bladders by implantation of acellular grafts. In this study, entire bladders where harvested from Sprague-Darley rats and subjected to a decellularization protocol to remove all the cells (103). The acellular bladders were referred to as BAMGs—bladder acellular matrix grafts. Using a second set of rats, a partial cystectomy was created to remove greater than 50% of the host bladder, and the BAMG was sutured onto the host bladder (103). The purpose of this surgery was not to support functional recovery of the host bladder. Instead, the objective of the study was to use the *in vivo* environment to fabricate artificial bladders. The host environment provides a range of physiological cues, growth factors for tissue development and maturation, and many other signals essential for tissue/organ function. In addition, the host tissue provides a potential source of cells that can infiltrate into the implanted BAMG and support scaffold cellularization. The BAMG was explanted after a period of several months, and the biological and functional performance metrics were evaluated and shown to be comparable to that of mammalian bladders. At the time of explanation, the tissue graft can be viewed as a bioengineered artificial bladder.

During the course of this book, we have studied several steps in the tissue fabrication process, including cell sourcing, scaffold cellularization, and bioreactor conditioning. In most of the examples that we have looked at thus far, these steps are conducted during controlled *in vitro* conditions; indeed, this is the most popular strategy to support the development of artificial tissue. However, in the current example, we see a novel application of the *in vivo* host environment to provide many of the cues required to support tissue development. This is advantageous, as the host environment has all the necessary signals to guide tissue development. However, the major limitation with this strategy is that the researcher does not have any control over the processing variables and cannot change any of the input variables.

In Vitro Organ Model for Bladder Tissue Engineering—Another very interesting study described the fabrication of hollow chamber artificial bladders using an

in vitro strategy. PGA was used as the biomaterial, and a large segment of PGA was used to create hollow chamber structures that resembled the urinary bladder. In order to increase the mechanical strength of the bioengineered structure, the PGA was coated with a liquefied copolymer that consisted of PLGA and methylene chloride (98). Cells were isolated from tissue specimens harvested from urinary bladders of dogs. The tissue specimens were subjected to a digestion protocol to isolate the SMCs and the epithelial cells, which were maintained in cell culture and expanded to increase the cell yield, as a very large number of cells were required to populate the PGA graft. The SMCs were seeded onto the exterior surface of the bioengineered graft, while the epithelial cells were seeded onto the luminal surface of the graft. The tissue graft was maintained in culture and then implanted *in vivo* in a dog model. A subtotal cystectomy was performed, sparing the trigone region, and the bioengineered bladder was implanted in recipient dogs. The functional performance of the implanted artificial bladder was assessed over a period of eleven months and compared with controls (grafts without any cells). The functional performance of the implanted artificial bladder was significantly greater than that of the controls' functional performance.

This study was clearly novel: it demonstrated the fabrication of artificial bladders using tissue engineering strategies. Many of the principles that we have covered in previous chapters were used in the development of this technology: biomaterial design and development, tissue fabrication, cell sourcing, and cell culture and scaffold cellularization.

Discussion of Organ Models for Bladder Tissue Engineering—In this section, we have looked at two examples for the fabrication of artificial bladders, one using an *in vivo* strategy and another using an *in vitro* method. These two examples serve to demonstrate the progression of research in the field of bladder tissue engineering from planar tissue specimens to complex artificial organs. The *in vivo* strategy is a novel method that makes use of internal physiological conditions to direct the growth and development of artificial bladders. The *in vitro* strategy is novel due to the ability to combine all the elements necessary for tissue fabrication toward the development of artificial bladders; this strategy demonstrates the utility of many of the tissue engineering principles that we have seen in earlier chapters and is clearly a seminal study in the field.

8.12 CLINICAL STUDY FOR BLADDER TISSUE ENGINEERING

There has been one reported clinical study using artificial bladders for the treatment of patients (104). This study is one of the seminal publications in the field of tissue engineering and proves the feasibility of bioengineered tissue (104). The current study provides significant impetus for the field and defines a path for the development of other artificial tissue and organ systems.

The clinical study was conducted by the same research group that developed the *in vitro* organ model for bladder tissue engineering that was described in the previous section. Therefore, there are several similarities between the work described

here and the work described in the previous section. The method used for the fabrication of scaffolds for the artificial bladder was similar to what was described in the previous section; the biomaterial was molded to form a hollow chamber structure which resembled the urinary bladder. In this study, different biomaterials were used. There were seven patients enrolled in this clinical study, and out of these seven patients, a collagen matrix was used in three patients, a collagen matrix wrapped with omentum was used in one patient, and a hybrid matrix of collagen and PGA wrapped with omentum was used in the final three patients. Three different scaffolding materials were used to bioengineer artificial bladders; this was a result of the progress made in understanding and optimizing fabrication technology for artificial bladders. The seven patients were recruited for this study over time rather than being recruited at the start of the clinical study. During this time, the researchers continued their efforts to optimize and improve artificial bladder fabrication and incorporated the refinements into the clinical study. It should be noted that the clinical study was not designed to compare different scaffolding materials for artificial bladder fabrication.

Autologous cells were obtained from a tissue biopsy from the patient's bladder and the SMCs and epithelial cells were isolated using a digestion protocol. The cells were maintained in culture and expanded to increase the cell yield. The SMCs were seeded on the exterior surface of the scaffold, and the epithelial cells were seeded on the luminal surface of the scaffold. The cell-seeded scaffold was maintained in a cell culture incubator for a period of 3–4 days prior to implantation. For surgical implantation in patients, the artificial bladders were anastomosized to the native bladders, and the patients were monitored for several months after the surgery. The results of the study demonstrated positive results both in terms of the functional performance of the implanted bladder and in terms of the histological assessment showing the distribution and alignment of the cells and ECM.

This study was a remarkable accomplishment and a cornerstone for the field of bladder tissue engineering and tissue engineering as a whole. In this study, many of the principles of tissue engineering were adapted, including biomaterials, tissue fabrication, cell isolation, culture and expansion, and scaffold cellularization. The principles of tissue engineering were used to fabricate artificial organs that were then implanted to help several patients and significantly improve their quality of life.

SUMMARY

Current State of the Art—Bladder tissue engineering is one of the more mature branches of the field; it has experienced proven clinical success. The clinical study remains a seminal publication and a cornerstone in the field of tissue engineering. There have been three primary sources of cells used to support the fabrication of artificial bladders. First, primary autologous cells have been used as a source of SMCs and epithelial cells. Second, autologous adipose-derived stem cells have been used, with the *in vivo* microenvironment supporting differentiation of the stem

cells to SMCs and epithelial cells. Third, infiltration of host cells into implanted acellullar grafts has also been used as a potential source of cells for bladder tissue engineering. Several biomaterials have been used to support the fabrication of artificial bladder tissue or artificial bladders, some of which include SIS, acellular grafts, and PLGA. In addition, cell sheeting engineering has been tested as a scaffold-free platform to support the development of artificial bladder tissue. Finally, we have looked at several tissue fabrication technologies that have been used to bioengineer artificial bladder tissue and entire organs. The transition has been from monolayer cell sheets to multilayer tissue grafts all the way to artificial bladders. Cell sheets have been used to coat the surface of gastric flaps prior to implantation, tissue patches have been engineered and shown to be effective in bladder augmentation, and entire bladders have been fabricated using *in vivo* and *in vitro* tissue engineering approaches.

Thoughts for Future Research—Considerable progress has been made in the field of bladder tissue engineering. However, there are several areas that have not been explored in great detail. The use of stem cells, including hES cells, iPS cells, and mesenchymal cells has not be explored in great detail; in addition, the signals that drive the differentiation of these cells toward bladder SMCs and epithelial cells need to be explored. There have not been many studies that look at the controlled vascularization and innervation of artificial bladder tissue under controlled *in vitro* culture conditions. Another area of research that has not received significant attention is the development of bioreactors to support the development and maturation of artificial bladder tissue. The normal function of the urinary bladder relies on SMC contraction, which results in changes in the pressure-volume relationship. There is a need to develop bioreactors that simulate these normal physiological conditions during the culture of artificial bladder tissue/organs.

PRACTICE QUESTIONS

1. Describe the structure of the urinary bladder, including the different cell types and the functions of these cells.
2. Discuss neurogenic bladder dysfunction as it relates to urinary bladder function. What are the clinical manifestations of neurogenic bladder dysfunction? What are some potential treatment modalities for this condition?
3. Describe the process of surgical bladder augmentation.
4. The process of surgical bladder augmentation relies upon autologous tissue grafts to expand the size of the urinary bladder. How can tissue engineering strategies be developed to improve this technique?
5. Describe the development of the urinary bladder during embryogenesis.
6. What can we learn about the development of the urinary bladder during embryogenesis that can be applied toward the fabrication of 3D artificial bladders?

7. During the course of this chapter, we provided a general description of the process of bioengineering artificial bladders and/or bladder tissue. Describe this process. If you had to add one additional step to the generic process, what would it be and why?

8. We have now studied two process schemes, one for the fabrication of artificial tracheas (Chapter 7) and one for the fabrication of artificial bladders (Chapter 8). What are some of the similarities between these two process schemes? What are some of the differences between these two process schemes?

9. Discuss the role of cell sheet engineering in bladder tissue engineering. Cell sheets have been used in conjunction with gastric segments for bladder reconstruction surgery; discuss the relative merits and weaknesses of this strategy.

10. Describe how cell sheet engineering can be used to fabricate complete artificial bladders.

11. In Chapter 4, we studied self-organization strategies for the fabrication of artificial tissue. Develop a strategy to fabricate artificial bladders or artificial bladder tissue using self-organization strategies.

12. Describe the use of SIS as a biomaterial. How is SIS fabricated? What are some of the properties of SIS that make it an attractive biomaterial? SIS is obtained from xenogenic sources; does this lead to immune rejection problems?

13. During our discussion of SIS in bladder tissue engineering, we described the use of SIS as a biomaterial for bladder augmentation and as a scaffold to support the fabrication of artificial bladder tissue. Discuss the relative advantages and disadvantages of each of the two strategies.

14. Develop a process in which SIS can be used to fabricate an entire artificial bladder.

15. Discuss the use of PLGA as a biomaterial in tissue engineering. What are some of the properties of the material that make it useful for tissue engineering? What fabrication technologies can be used for the fabrication of 3D scaffolds using PLGA?

16. Discuss the use of PLGA for bladder augmentation with and without cells. What are the relative advantages of each of the two methods?

17. Develop a process in which PLGA can be used to fabricate 3D artificial bladder tissue.

18. Discuss the relative advantages and disadvantages of acellular grafts for bladder tissue engineering.

19. What are some potential uses of bioreactors during the fabrication of artificial bladders? Design a bioreactor to support the fabrication and/or culture of artificial bladders.

20. What is the potential role of perfusion systems during the culture of artificial bladders? Design a perfusion system to support the culture of artificial bladders.

REFERENCES

1. Preusser S, Diener PA, Schmid HP, Leippold T. Submucosal endocervicosis of the bladder: an ectopic, glandular structure of Mullerian origin. Scand. J. Urol. Nephrol. 2008;42(1):88–90.
2. Dorschner W, Stolzenburg JU, Neuhaus J. Structure and function of the bladder neck. Adv. Anat. Embryol. Cell Biol. 2001;159:III-109.
3. Gabella G. Structure of the intramural nerves of the rat bladder. J. Neurocytol. 1999 Aug;28(8):615–37.
4. Gosling JA. The structure of the bladder neck, urethra and pelvic floor in relation to female urinary continence. Int. Urogynecol. J. Pelvic. Floor. Dysfunct. 1996;7(4): 177–8.
5. Gabella G, Uvelius B. Urinary bladder of rat: fine structure of normal and hypertrophic musculature. Cell Tissue Res. 1990 Oct;262(1):67–79.
6. Holm-Bentzen M, Ammitzboll T. Structure and function of glycosaminoglycans in the bladder. Ann. Urol. (Paris) 1989;23(2):167–8.
7. Wade RH, Brisson A. Three-dimensional structure of bladder membrane protein. Ultramicroscopy 1984;13(1–2):47–56.
8. Gosling J. The structure of the bladder and urethra in relation to function. Urol. Clin. North Am. 1979 Feb;6(1):31–8.
9. Gosling JA, Dixon JS, Dunn M. The structure of the rabbit urinary bladder after experimental distension. Invest Urol. 1977 Mar;14(5):386–9.
10. Ellis DJ. The bladder neck: a theory of its structure and function. Br. J. Urol. 1972 Dec;44(6):727–8.
11. Hoyes AD, Ramus NI, Martin BG. Fine structure of the epithelium of the human fetal bladder. J. Anat. 1972 Apr;111(Pt 3):415–25. PMCID:PMC1271131.
12. Bro-Rasmussen F, Sorensen AH, Bredahl E, Kelstrup A. The Structure and Function of the Urinary Bladder. Urol. Int. 1965;19:280–95.
13. Brading AF, Greenland JE, Mills IW, McMurray G, Symes S. Blood supply to the bladder during filling. Scand. J. Urol. Nephrol. Suppl 1999;201:25–31.
14. Hossler FE, Monson FC. Structure and blood supply of intrinsic lymph nodes in the wall of the rabbit urinary bladder–studies with light microscopy, electron microscopy, and vascular corrosion casting. Anat. Rec. 1998 Nov;252(3):477–84.
15. Irwin P, Galloway NT. Impaired bladder perfusion in interstitial cystitis: a study of blood supply using laser Doppler flowmetry. J. Urol. 1993 Apr;149(4):890–2.
16. Michels NA. Variations in the blood-supply of the liver, gall bladder, stomach, duodenum, pancreas and spleen; 200 dissections. Am. J. Med. Sci. 1948 Jul;216(1):115.

17. Clemens JQ. Basic bladder neurophysiology. Urol. Clin. North Am. 2010 Nov;37(4): 487–94.
18. Campioni P, Goletti S, Palladino F, Nanni M, Napoli M, Valentini AL. The neurogenic bladder: anatomy and neurophysiology. Rays 2002 Apr;27(2):107–14.
19. Yeates WK. Neurophysiology of the bladder. Paraplegia 1974 Aug;12(2):73–82.
20. Agarwal SK, Bagli DJ. Neurogenic bladder. Indian J. Pediatr. 1997 May;64(3): 313–26.
21. Madersbacher HG. Neurogenic bladder dysfunction. Curr. Opin. Urol. 1999 Jul;9(4): 303–7.
22. Nijman RJ. Neurogenic and non-neurogenic bladder dysfunction. Curr. Opin. Urol. 2001 Nov;11(6):577–83.
23. Salvaggio E, Arces L, Rendeli C. Clinical patterns of neurogenic bladder. Rays 2002 Apr;27(2):115–20.
24. Verpoorten C, Buyse GM. The neurogenic bladder: medical treatment. Pediatr. Nephrol. 2008 May;23(5):717–25. PMCID:PMC2275777.
25. Bauer SB. Neurogenic bladder: etiology and assessment. Pediatr. Nephrol. 2008 Apr;23(4):541–51. PMCID:PMC2259256.
26. MacLellan DL. Management of pediatric neurogenic bladder. Curr. Opin. Urol. 2009 Jul;19(4):407–11.
27. 27.Cameron AP. Pharmacologic therapy for the neurogenic bladder. Urol. Clin. North Am. 2010 Nov;37(4):495–506.
28. Westney OL. The neurogenic bladder and incontinent urinary diversion. Urol. Clin. North Am. 2010 Nov;37(4):581–92.
29. Klausner AP, Steers WD. The neurogenic bladder: an update with management strategies for primary care physicians. Med. Clin. North Am. 2011 Jan;95(1):111–20.
30. McGuire EJ. Urodynamics of the neurogenic bladder. Urol. Clin. North Am. 2010 Nov;37(4):507–16.
31. Gormley EA. Urologic complications of the neurogenic bladder. Urol. Clin. North Am. 2010 Nov;37(4):601–7.
32. Dorsher PT, McIntosh PM. Neurogenic bladder. Adv. Urol. 2012;2012:816274. PMCID:PMC3287034.
33. Yeates WK. Neurophysiology of the bladder. Paraplegia 1974 Aug;12(2):73–82.
34. Halverstadt DB, Leadbetter WF. Electrical stimulation of the human bladder: experience in three patients with hypotonic neurogenic bladder dysfunction. Br. J. Urol. 1968 Apr;40(2):175–82.
35. Hald T. Neurogenic dysfunction of the urinary bladder. An experimental and clinical study with special reference to the ability of electrical stimulation to establish voluntary micturition. Dan. Med. Bull. 1969 Jun;16:Suppl.
36. Tanagho EA, Schmidt RA. Electrical stimulation in the clinical management of the neurogenic bladder. J. Urol. 1988 Dec;140(6):1331–9.
37. Madias JE. Electrocardiographic artifact induced by an electrical stimulator implanted for management of neurogenic bladder. J. Electrocardiol. 2008 Sep;41(5):401–3.
38. Tellenbach M, Schneider M, Mordasini L, Thalmann GN, Kessler TM. Transcutaneous electrical nerve stimulation: an effective treatment for refractory non-neurogenic overactive bladder syndrome? Urol: World J; 2012 May 24.

39. Radziszewski K. Outcomes of electrical stimulation of the neurogenic bladder: Results of a two-year follow-up study. NeuroRehabilitation 2013 Jan 1;32(4):867–73.
40. Guys JM, Haddad M, Planche D, Torre M, Louis-Borrione C, Breaud J. Sacral neuromodulation for neurogenic bladder dysfunction in children. J. Urol. 2004 Oct; 172(4 Pt 2):1673–6.
41. Hohenfellner M, Humke J, Hampel C, Dahms S, Matzel K, Roth S, Thuroff JW, Schultz-Lampel D. Chronic sacral neuromodulation for treatment of neurogenic bladder dysfunction: long-term results with unilateral implants. Urology 2001 Dec;58(6):887–92.
42. Gurocak S, Nuininga J, Ure I, De Gier RP, Tan MO, Feitz W. Bladder augmentation: Review of the literature and recent advances. Indian J. Urol. 2007 Oct;23(4):452–7. PMCID:PMC2721579.
43. Babu R, Ragoori D. Bladder augmentation: Distal ureterocystoplasty with proximal ureteric reimplantation: A novel technique. J. Indian Assoc. Pediatr. Surg. 2012 Oct;17(4):165–7. PMCID:PMC3518995.
44. Metcalfe PD, Rink RC. Bladder augmentation: complications in the pediatric population. Curr. Urol. Rep. 2007 Mar;8(2):152–6.
45. Gurocak S, De Gier RP, Feitz W. Bladder augmentation without integration of intact bowel segments: critical review and future perspectives. J. Urol. 2007 Mar;177(3):839–44.
46. Schaefer M, Kaiser A, Stehr M, Beyer HJ. Bladder augmentation with small intestinal submucosa leads to unsatisfactory long-term results. Urol: J. Pediatr. 2013 Jan 15.
47. Escudero RM, Patino GE, Fernandez ER, Gil MJ, Garcia EL, Alonso AH, Pinies GO, Sanchez JP, Fernandez CH. Bladder augmentation using the gastrointestinal tract. Indication, follow up and complications. Arch. Esp. Urol. 2011 Dec;64(10):953–9.
48. Stein R, Kamal MM, Rubenwolf P, Ziesel C, Schroder A, Thuroff JW. Bladder augmentation using bowel segments (enterocystoplasty). BJU. Int. 2012 Oct;110(7): 1078–94.
49. Quiroz-Guerrero J, Badillo M, Munoz N, Anaya J, Rico G, Maldonado-Valadez R. Bladder augmentation in a young adult female exstrophy patient with associated omphalocele: an extremely unusual case. J. Pediatr. Urol. 2009 Aug;5(4):330–2.
50. Daher P, Zeidan S, Riachy E, Iskandarani F. Bladder augmentation and/or continent urinary diversion: 10-year experience. Eur. J. Pediatr. Surg. 2007 Apr;17(2):119–23.
51. Stein R, Schroder A, Thuroff JW. Bladder augmentation and urinary diversion in patients with neurogenic bladder: non-surgical considerations. J. Pediatr. Urol. 2012 Apr;8(2):145–52.
52. Sajadi KP, Goldman HB. Bladder augmentation and urinary diversion for neurogenic LUTS: current indications. Curr. Urol. Rep. 2012 Oct;13(5):389–93.
53. Rodo JS, Caceres FA, Lerena JR, Rossy E. Bladder augmentation and artificial sphincter implantation: urodynamic behavior and effects on continence. J. Pediatr. Urol. 2008 Feb;4(1):8–13.
54. Hannema SE, Hughes IA. Regulation of Wolffian duct development. Horm. Res. 2007;67(3):142–51.
55. Staack A, Donjacour AA, Brody J, Cunha GR, Carroll P. Mouse urogenital development: a practical approach. Differentiation 2003 Sep;71(7):402–13.

56. Cuckow PM, Nyirady P, Winyard PJ. Normal and abnormal development of the urogenital tract. Prenat. Diagn. 2001 Nov;21(11):908–16.

57. Shiroyanagi Y, Yamato M, Yamazaki Y, Toma H, Okano T. Transplantable urothelial cell sheets harvested noninvasively from temperature-responsive culture surfaces by reducing temperature. Tissue Eng. 2003 Oct;9(5):1005–12.

58. Watanabe E, Yamato M, Shiroyanagi Y, Tanabe K, Okano T. Bladder augmentation using tissue-engineered autologous oral mucosal epithelial cell sheets grafted on demucosalized gastric flaps. Transplantation 2011 Apr 15;91(7):700–6.

59. Kang KN, Lee JY, Kim dY, Lee BN, Ahn HH, Lee B, Khang G, Park SR, Min BH, Kim JH, et al. Regeneration of completely transected spinal cord using scaffold of poly(D,L-lactide-co-glycolide)/small intestinal submucosa seeded with rat bone marrow stem cells. Tissue Eng. Part A 2011 Sep;17(17–18):2143–52.

60. Schnoeller TJ, de PR, Hefty R, Jentzmik F, Waalkes S, Zengerling F, Schrader M, Schrader AJ. Partial nephrectomy using porcine small intestinal submucosa. World J. Surg. Oncol. 2011;9:126. PMCID:PMC3233505.

61. Palminteri E, Berdondini E, Fusco F, De NC, Salonia A. Long-term results of small intestinal submucosa graft in bulbar urethral reconstruction. Urology 2012 Mar;79(3):695–701.

62. Ma L, Yang Y, Sikka SC, Kadowitz PJ, Ignarro LJ, Abdel-Mageed AB, Hellstrom WJ. Adipose tissue-derived stem cell-seeded small intestinal submucosa for tunica albuginea grafting and reconstruction. Proc. Natl. Acad. Sci. U.S.A 2012 Feb 7; 109(6):2090–5. PMCID:PMC3277542.

63. Lee AJ, Chung WH, Kim DH, Lee KP, Suh HJ, Do SH, Eom KD, Kim HY. Use of canine small intestinal submucosa allograft for treating perineal hernias in two dogs. J. Vet. Sci. 2012 Sep;13(3):327–30. PMCID:PMC3467410.

64. Andree B, Bar A, Haverich A, Hilfiker A. Small intestinal submucosa segments as matrix for tissue engineering: review. Tissue Eng. Part B Rev 2013 Aug;19(4):279–91.

65. Witt RG, Raff G, Van GJ, Rodgers-Ohlau M, Si MS. Short-term experience of porcine small intestinal submucosa patches in paediatric cardiovascular surgery. Eur. J. Cardiothorac. Surg. 2013 Jul;44(1):72–6.

66. Song Z, Peng Z, Liu Z, Yang J, Tang R, Gu Y. Reconstruction of abdominal wall musculofascial defects with small intestinal submucosa scaffolds seeded with tenocytes in rats. Tissue Eng. Part A 2013 Jul;19(13–14):1543–53. PMCID:PMC3665322.

67. Yi JS, Lee HJ, Lee HJ, Lee IW, Yang JH. Rat peripheral nerve regeneration using nerve guidance channel by porcine small intestinal submucosa. J. Korean Neurosurg. Soc. 2013 Feb;53(2):65–71. PMCID:PMC3611061.

68. Rossetto VJ, da Mota LS, Rocha NS, Miot HA, Grandi F, Brandao CV. Grafts of porcine small intestinal submucosa seeded with cultured homologous smooth muscle cells for bladder repair in dogs. Acta Vet. Scand. 2013; 55:39. PMCID:PMC3663814.

69. Lai JY, Chang PY, Lin JN. Bladder autoaugmentation using various biodegradable scaffolds seeded with autologous smooth muscle cells in a rabbit model. J. Pediatr. Surg. 2005 Dec;40(12):1869–73.

70. Kropp BP, Eppley BL, Prevel CD, Rippy MK, Harruff RC, Badylak SF, Adams MC, Rink RC, Keating MA. Experimental assessment of small intestinal submucosa as a bladder wall substitute. Urology 1995 Sep;46(3):396–400.

71. Hawley AE, Illum L, Davis SS. Preparation of biodegradable, surface engineered PLGA nanospheres with enhanced lymphatic drainage and lymph node uptake. Pharm. Res. 1997 May;14(5):657–61.
72. Nakanishi Y, Chen G, Komuro H, Ushida T, Kaneko S, Tateishi T, Kaneko M. Tissue-engineered urinary bladder wall using PLGA mesh-collagen hybrid scaffolds: a comparison study of collagen sponge and gel as a scaffold. J. Pediatr. Surg. 2003 Dec;38(12):1781–4.
73. Baek CH, Ko YJ. Characteristics of tissue-engineered cartilage on macroporous biodegradable PLGA scaffold. Laryngoscope 2006 Oct;116(10):1829–34.
74. Jeong SI, Kim SY, Cho SK, Chong MS, Kim KS, Kim H, Lee SB, Lee YM. Tissue-engineered vascular grafts composed of marine collagen and PLGA fibers using pulsatile perfusion bioreactors. Biomaterials 2007 Feb;28(6):1115–22.
75. Holgado MA, Cozar-Bernal MJ, Salas S, Arias JL, Alvarez-Fuentes J, Fernandez-Arevalo M. Protein-loaded PLGA microparticles engineered by flow focusing: physicochemical characterization and protein detection by reversed-phase HPLC. Int. J. Pharm. 2009 Oct 1;380(1–2):147–54.
76. Kang SW, Lee SJ, Kim JS, Choi EH, Cha BH, Shim JH, Cho DW, Lee SH. Effect of a scaffold fabricated thermally from acetylated PLGA on the formation of engineered cartilage. Macromol. Biosci. 2011 Feb 11;11(2):267–74.
77. Enlow EM, Luft JC, Napier ME, DeSimone JM. Potent engineered PLGA nanoparticles by virtue of exceptionally high chemotherapeutic loadings. Nano. Lett. 2011 Feb 9;11(2):808–13. PMCID:PMC3122105.
78. Jain AK, Das M, Swarnakar NK, Jain S. Engineered PLGA nanoparticles: an emerging delivery tool in cancer therapeutics. Crit Rev. Ther. Drug Carrier Syst. 2011;28(1):1–45.
79. Ungaro F, d'Angelo I, Miro A, La Rotonda MI, Quaglia F. Engineered PLGA nano- and micro-carriers for pulmonary delivery: challenges and promises. J. Pharm. Pharmacol 2012 Sep;64(9):1217–35.
80. Patrick CW Jr, Chauvin PB, Hobley J, Reece GP. Preadipocyte seeded PLGA scaffolds for adipose tissue engineering. Tissue Eng. 1999 Apr;5(2):139–51.
81. Holy CE, Cheng C, Davies JE, Shoichet MS. Optimizing the sterilization of PLGA scaffolds for use in tissue engineering. Biomaterials 2001 Jan;22(1):25–31.
82. Koegler WS, Griffith LG. Osteoblast response to PLGA tissue engineering scaffolds with PEO modified surface chemistries and demonstration of patterned cell response. Biomaterials 2004 Jun;25(14):2819–30.
83. Hwang CM, Khademhosseini A, Park Y, Sun K, Lee SH. Microfluidic chip-based fabrication of PLGA microfiber scaffolds for tissue engineering. Langmuir 2008 Jun 1;24(13):6845–51.
84. Tan H, Wu J, Lao L, Gao C. Gelatin/chitosan/hyaluronan scaffold integrated with PLGA microspheres for cartilage tissue engineering. Acta Biomater. 2009 Jan;5(1): 328–37.
85. Jose MV, Thomas V, Johnson KT, Dean DR, Nyairo E. Aligned PLGA/HA nanofibrous nanocomposite scaffolds for bone tissue engineering. Acta Biomater. 2009 Jan;5(1):305–15.
86. Baker SC, Rohman G, Southgate J, Cameron NR. The relationship between the mechanical properties and cell behaviour on PLGA and PCL scaffolds for bladder tissue engineering. Biomaterials 2009 Mar;30(7):1321–8.

87. van EF, Saris DB, Fedorovich NE, Kruyt MC, Willems WJ, Verbout AJ, Martens AC, Dhert WJ, Creemers L. In vivo matrix production by bone marrow stromal cells seeded on PLGA scaffolds for ligament tissue engineering. Tissue Eng. Part A 2009 Oct;15(10):3109–17.
88. Ngiam M, Liao S, Patil AJ, Cheng Z, Chan CK, Ramakrishna S. The fabrication of nano-hydroxyapatite on PLGA and PLGA/collagen nanofibrous composite scaffolds and their effects in osteoblastic behavior for bone tissue engineering. Bone 2009 Jul;45(1):4–16.
89. Sahoo S, Toh SL, Goh JC. PLGA nanofiber-coated silk microfibrous scaffold for connective tissue engineering. J. Biomed. Mater. Res. B Appl. Biomater. 2010 Oct;95(1):19–28.
90. Andreas K, Zehbe R, Kazubek M, Grzeschik K, Sternberg N, Baumler H, Schubert H, Sittinger M, Ringe J. Biodegradable insulin-loaded PLGA microspheres fabricated by three different emulsification techniques: investigation for cartilage tissue engineering. Acta Biomater. 2011 Apr;7(4):1485–95.
91. Han J, Lazarovici P, Pomerantz C, Chen X, Wei Y, Lelkes PI. Co-electrospun blends of PLGA, gelatin, and elastin as potential nonthrombogenic scaffolds for vascular tissue engineering. Biomacromolecules 2011 Feb 14;12(2):399–408.
92. Go DP, Palmer JA, Gras SL, O'Connor AJ. Coating and release of an anti-inflammatory hormone from PLGA microspheres for tissue engineering. J. Biomed. Mater. Res. A 2011 Nov 29.
93. Takechi M, Ohta K, Ninomiya Y, Tada M, Minami M, Takamoto M, Ohta A, Nakagawa T, Fukui A, Miyamoto Y, et al. 3-dimensional composite scaffolds consisting of apatite-PLGA-atelocollagen for bone tissue engineering. Dent. Mater. J. 2012;31(3):465–71.
94. Chang NJ, Jhung YR, Yao CK, Yeh ML. Hydrophilic gelatin and hyaluronic acid-treated PLGA scaffolds for cartilage tissue engineering. J. Appl. Biomater. Funct. Mater. 2013;11(1):e45–e52.
95. Horst M, Madduri S, Milleret V, Sulser T, Gobet R, Eberli D. A bilayered hybrid microfibrous PLGA–acellular matrix scaffold for hollow organ tissue engineering. Biomaterials 2013 Feb; 34(5):1537–45.
96. Wang PY, Wu TH, Tsai WB, Kuo WH, Wang MJ. Grooved PLGA films incorporated with RGD/YIGSR peptides for potential application on skeletal muscle tissue engineering. Colloids Surf. B Biointerfaces. 2013 Oct 1;110:88–95.
97. Jayo MJ, Jain D, Wagner BJ, Bertram TA. Early cellular and stromal responses in regeneration versus repair of a mammalian bladder using autologous cell and biodegradable scaffold technologies. J. Urol. 2008 Jul;180(1):392–7.
98. Oberpenning F, Meng J, Yoo JJ, Atala A. De novo reconstitution of a functional mammalian urinary bladder by tissue engineering. Nat. Biotechnol. 1999 Feb;17(2):149–55.
99. Reddy PP, Barrieras DJ, Wilson G, Bagli DJ, McLorie GA, Khoury AE, Merguerian PA. Regeneration of functional bladder substitutes using large segment acellular matrix allografts in a porcine model. J. Urol. 2000 Sep;164(3 Pt 2):936–41.
100. Merguerian PA, Reddy PP, Barrieras DJ, Wilson GJ, Woodhouse K, Bagli DJ, McLorie GA, Khoury AE. Acellular bladder matrix allografts in the regeneration of functional bladders: evaluation of large-segment (>24 cm) substitution in a porcine model. BJU. Int. 2000 May;85(7):894–8.

101. Cheng HL, Loai Y, Farhat WA. Monitoring tissue development in acellular matrix-based regeneration for bladder tissue engineering: multiexponential diffusion and T2* for improved specificity. NMR Biomed. 2012 Mar;25(3):418–26.
102. Zhu WD, Xu YM, Feng C, Fu Q, Song LJ, Cui L. Bladder reconstruction with adipose-derived stem cell-seeded bladder acellular matrix grafts improve morphology composition. World J. Urol. 2010 Aug;28(4):493–8.
103. Piechota HJ, Dahms SE, Nunes LS, Dahiya R, Lue TF, Tanagho EA. In vitro functional properties of the rat bladder regenerated by the bladder acellular matrix graft. J. Urol. 1998 May;159(5):1717–24.
104. Atala A, Bauer SB, Soker S, Yoo JJ, Retik AB. Tissue-engineered autologous bladders for patients needing cystoplasty. Lancet 2006 Apr 15;367(9518):1241–6.