Tissue engineering of human bladder

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There are a number of conditions of the bladder that can lead to loss of function. Many of these require reconstructive procedures. However, current techniques may lead to a number of complications. Replacement of bladder tissues with functionally equivalent ones created in the laboratory could improve the outcome of reconstructive surgery. A review of the literature was conducted using PubMed to identify studies that provide evidence that tissue engineering techniques may be useful in the development of alternatives to current methods of bladder reconstruction. A number of animal studies and several clinical experiences show that it is possible to reconstruct the bladder using tissues and neo-organs produced in the laboratory. Materials that could be used to create functionally equivalent urologic tissues in the laboratory, especially non-autologous cells that have the potential to reject have many technical limitations. Current research suggests that the use of biomaterial-based, bladder-shaped scaffolds seeded with autologous urothelial and smooth muscle cells is currently the best option for bladder tissue engineering. Further research to develop novel biomaterials and cell sources, as well as information gained from developmental biology, signal transduction studies and studies of the wound healing response would be beneficial.

Keywords: bladder/tissue engineering/cystectomy/reconstruction/regenerative medicine/stem cells/biomaterials

Accepted: January 11, 2011
Introduction

Congenital disorders, cancer, trauma, infection, inflammation, iatrogenic injuries or other conditions of the genitourinary system can lead to bladder damage. Most of these situations require eventual reconstructive procedures. These procedures can be performed with native non-urologic tissues (skin, gastrointestinal segments or mucosa), heterologous tissues or substances (bovine collagen) or artificial materials (silicone, polyurethane, Teflon). Currently, gastrointestinal segments are most commonly used as tissues for bladder replacement or repair. However, gastrointestinal tissues are designed to absorb specific solutes, whereas bladder tissue is designed for the excretion of these same solutes. As a result, when gastrointestinal tissue is placed within the urinary tract, multiple complications may ensue. These include infection, metabolic disturbances, urolithiasis, perforation, increased mucus production and malignancy.1–4 Because of the problems encountered with the use of gastrointestinal segments, numerous investigators have attempted alternative reconstructive procedures for bladder replacement or repair. These include autologous5,6 and ureterocystoplasty.7–9 In addition, novel methods for bladder reconstruction based on regenerative medicine, such as cell transplantation and tissue engineering, are being explored. This review focuses specifically on these novel regenerative medicine strategies for bladder reconstruction.

The basics of tissue engineering

Tissue engineering employs aspects of cell biology and transplantation, materials science and biomedical engineering to develop biological substitutes that can restore and maintain the normal function of damaged tissues and organs. These include injection of functional cells into a non-functional site to stimulate regeneration and the use of biocompatible materials to create new tissues and organs. These biomaterials can be natural or synthetic matrices, often termed scaffolds, which encourage the body’s natural ability to repair itself and assist in determination of the orientation and direction of new tissue growth. Often, tissue engineering uses a combination of both of these techniques. For example, biomaterial matrices seeded with cells can be implanted into the body to encourage the growth or regeneration of functional tissue.

Biomaterials used in genitourinary tissue construction

Synthetic materials have been used widely for urologic reconstruction. Silicone prostheses have been used for the treatment of urinary
incontinence with the artificial urinary sphincter and detachable balloon system, for treatment of vesicoureteral reflux with silicone microparticles, and for impotence with penile prostheses.\textsuperscript{10–13} There has also been a major effort directed toward the construction of artificial bladders made with silicone. In some disease states, such as urinary incontinence or vesicoureteral reflux, artificial agents (Teflon paste, glass microparticles) have been used as injectable bulking substances; however, these substances are not entirely biocompatible.\textsuperscript{14}

For regenerative medicine purposes, there are clear advantages to using degradable, biocompatible materials that can function as cell delivery vehicles, and/or provide the structural parameters needed for tissue replacement. Biomaterials in genitourinary regenerative medicine function as an artificial extracellular matrix (ECM) and elicit biologic and mechanical functions of native ECM found in tissues in the body. Native ECM brings cells together into tissue, controls the tissue structure and regulates the cell phenotype.\textsuperscript{15} Biomaterials facilitate the localization and delivery of cells and/or bioactive factors (e.g. cell adhesion peptides, growth factors) to desired sites in the body, define a three-dimensional space for the formation of new tissues with appropriate structure, and guide the development of new tissues with appropriate function.\textsuperscript{16} Direct injection of cell suspensions without biomaterial matrices has been used in some cases,\textsuperscript{17,18} but it is difficult to control the localization of transplanted cells. In addition, the majority of mammalian cell types are anchorage dependent and will die if not provided with an appropriate cell adhesion substrate.

**Design and selection of biomaterials**

The design and selection of a biomaterial for use in regenerative medicine is critical for the proper development of engineered genitourinary tissues. The selected biomaterial must be capable of controlling the structure and function of the engineered tissue in a predesigned manner by interacting with transplanted cells and/or host cells. In addition, it should be biocompatible, able to promote cellular interaction and tissue development, and it should possess the proper mechanical and physical properties required for tissue support and function in the body site of interest.

Appropriate biomaterials should be biodegradable and bioresorbable to support the reconstruction of a completely normal tissue without inflammation. Thus, the degradation rate and the concentration of degradation products in the tissues surrounding the implant must be maintained at a tolerable level.\textsuperscript{19} Such behavior avoids the risk of inflammatory or foreign-body responses that is often associated with the permanent presence of a foreign material in the body.
In addition, the biomaterial should provide appropriate regulation of cell behavior (e.g. adhesion, proliferation, migration, differentiation) in order to promote the development of functional new tissue. Cell behavior in engineered tissues is regulated by multiple interactions with the microenvironment, including interactions with cell-adhesion ligands and with soluble growth factors. Cell-adhesion-promoting factors (e.g. Arg–Gly–Asp [RGD]) can be presented by the biomaterial itself or incorporated into the biomaterial in order to control cell behavior through ligand-induced cell receptor signaling processes. As an example, a scaffold used to create an engineered bladder must be able to support the adhesion and proliferation of a number of cell types, including urothelial cells on the luminal side and smooth muscle cells surrounding the urothelial barrier, and it must be able to direct proper tissue development in order to form a functional bladder. In order to accomplish this, composite scaffolds consisting of both collagen and synthetic materials have been produced for hollow organ engineering.

In vivo, the biomaterials must provide temporary mechanical support sufficient to withstand forces exerted by the surrounding tissue and maintain a potential space for tissue development. In the case of bladder replacement, the biomaterial used to form the engineered organ must be able to withstand forces resulting from urine storage and filling/emptying. In addition, the biomaterial must be able to withstand the forces exerted on it by the pelvic muscles as the patient goes about daily activities. The mechanical support of the biomaterials should be maintained until the engineered tissue has sufficient mechanical integrity to support itself. This can be achieved by an appropriate choice of mechanical and degradative properties of the biomaterials.

Finally, the chosen biomaterial must have properties that allow it to be processed into specific configurations. For example, it must be molded into a tubular shape for urethral replacement, or it must be shaped into a hollow, spherical configuration for bladder replacement. A large ratio of surface area to volume is often desirable to allow the delivery of a high density of cells. A high porosity, interconnected pore structure with specific pore sizes promotes tissue ingrowth from the surrounding host tissue. Several techniques, such as electrospinning, have been developed, and they allow precise control of porosity, pore size and pore structure.

Types of biomaterials
Generally, three classes of biomaterials have been used for engineering of genitourinary tissues: naturally derived materials, such as collagen and alginate; acellular tissue matrices, such as bladder submucosa (BSM) and small-intestinal submucosa (SIS) and synthetic polymers, such as polyglycolic acid (PGA), polylactic acid (PLA) and
poly(lactic-co-glycolic acid) (PLGA). These classes of biomaterials have been tested to determine their biocompatibility with primary human urothelial and bladder muscle cells. Naturally derived materials and acellular tissue matrices have the potential advantage of biologic recognition. However, synthetic polymers can be produced quickly and reproducibly on a large scale with controlled properties of strength, degradation rate and microstructure.

Collagen is the most abundant and ubiquitous structural protein in the body, and it may be readily purified from both animal and human tissues with an enzyme treatment and salt/acid extraction. Collagen has long been known to exhibit minimal inflammatory and antigenic responses, and it has been approved by the US Food and Drug Administration (FDA) for many types of medical applications, including wound dressings and artificial skin. Intermolecular cross-linking reduces the degradation rate by making the collagen molecules less susceptible to enzymatic attack. Intermolecular cross-linking can be accomplished by various physical (e.g. ultraviolet radiation, dehydrothermal treatment) or chemical (e.g. glutaraldehyde, formaldehyde, carbodiimides) techniques. Collagen contains cell-adhesion domain sequences (e.g. RGD) that exhibit specific cellular interactions. This may help to retain the phenotype and activity of many types of cells, including fibroblasts and chondrocytes. This material can be processed into a wide variety of structures such as sponges, fibers and films.

Alginate, a polysaccharide isolated from seaweed, has been used as an injectable cell delivery vehicle and a cell immobilization matrix (Lim and Sun, 1980) owing to its gentle gelling properties in the presence of divalent ions such as calcium. Alginate is a family of copolymers of D-mannuronate and L-guluronate. The physical and mechanical properties of alginate gel are strongly correlated with the proportion and length of the polyguluronate block in the alginate chains. Efforts have been made to synthesize biodegradable alginate hydrogels with mechanical properties that are controllable in a wide range by intermolecular covalent cross-linking and with cell-adhesion peptides coupled to their backbones.

Recently, natural materials such as alginate and collagen have been used as ‘bio-inks’ in a newly developed bioprinting technique based on inkjet technology. Using this technology, these scaffold materials can be ‘printed’ into a desired scaffold shape using a modified inkjet printer. In addition, several groups have shown that living cells can also be printed using this technology. This exciting technique can be modified so that a three-dimensional construct containing a precise arrangement of cells, growth factors and extracellular matrix material
can be printed.⁴⁷–⁴⁹ Such constructs may eventually be implanted into a host to serve as the backbone for a new tissue or organ.

Acellular tissue matrices are collagen-rich matrices prepared by removing cellular components from tissues. The most common tissue that has been used for this purpose has been bladder tissue. The matrices are prepared by removing the cellular material from a segment of bladder tissue using mechanical and chemical processes.⁵⁰–⁵³ The resulting matrix can be used alone or seeded with cells. The matrices slowly degrade after implantation and are replaced and remodeled by ECM proteins synthesized and secreted by transplanted or ingrowing cells. Acellular tissue matrices support cell ingrowth and regeneration of several genitourinary tissue types, including urethra and bladder, with no evidence of immunogenic rejection.⁵³,⁵⁴ Because the structures of the proteins (e.g. collagen, elastin) in acellular matrices are well conserved and normally arranged, the mechanical properties of the acellular matrices are not significantly different from those of native BSM.⁵⁰ Polyesters of naturally occurring α-hydroxy acids, including PGA, PLA and PLGA, are widely used in regenerative medicine. These polymers have gained FDA approval for human use in a variety of applications, including sutures.⁵⁵ The degradation products of PGA, PLA and PLGA are non-toxic, natural metabolites that are eventually eliminated from the body in the form of carbon dioxide and water.⁵⁵ Because these polymers are thermoplastics, they can easily be formed into a three-dimensional scaffold with a desired microstructure, gross shape and dimension by various techniques, including molding, extrusion,⁵⁶ solvent casting,⁵⁷ phase separation techniques and gas-foaming techniques.⁵⁸ More recently, techniques such as electrospinning have been used to quickly create highly porous scaffolds in various conformations.²⁸–³⁰,⁵⁹

Many applications in genitourinary regenerative medicine require a scaffold with high porosity and a high ratio of surface area to volume. This need has been addressed by processing biomaterials into configurations of fiber meshes and porous sponges using the techniques described previously. A drawback of the synthetic polymers is lack of biologic recognition. As an approach toward incorporating cell-recognition domains into these materials, copolymers with amino acids have been synthesized.²²,²³,⁶⁰ Other biodegradable synthetic polymers, including poly(anhydrides) and poly(ortho-esters), can also be used to fabricate scaffolds for genitourinary regenerative medicine with controlled properties.⁶¹ In addition, composite scaffolds consisting of both natural and synthetic materials have been developed and may be useful in genitourinary tissue engineering. In particular, these scaffolds may be useful for engineering organs that are composed of layers of cells, such as the bladder (urothelial layer surrounded by smooth muscle cells).²⁴
Nanotechnology, which is the use of small molecules that have distinct properties on a small scale, has been used to create ‘smart biomaterials’ for regenerative medicine.\(^6^2,^6^3\) Nanoscaffolds have been manufactured specifically for bladder applications.\(^6^4\) The manufacturing of nanostructured biomaterials has also led to enhanced cell alignment and tissue formation.\(^2^8\)

**Cells for urogenital tissue engineering applications**

Often, when cells are used for tissue engineering, donor tissue is removed and dissociated into individual cells, which are implanted directly into the host or expanded in culture, attached to a support matrix and then implanted. The implanted tissue can be heterologous, allogeneic or autologous. Ideally, this approach allows lost tissue function to be restored or replaced *in toto* with limited complications.\(^6^5–^7^0\)

Autologous cells are the ideal choice, as their use circumvents many of the inflammatory and rejection issues associated with a non-self donor. In the past, one of the limitations of applying cell-based regenerative medicine techniques to organ replacement was the inherent difficulty of growing certain human cell types in large quantities. However, the discovery of native targeted progenitor cells in virtually every organ of the body has led to improved culture techniques that have overcome this problem for a number of cell types. Native targeted progenitor cells are tissue-specific unipotent cells derived from most organs. By noting the location of the progenitor cells, as well as by exploring the conditions that promote differentiation and/or self-renewal, it has been possible to overcome some of the obstacles that limit cell expansion *in vitro*. For example, urothelial cell culture has been improved in this way. Urothelial cells could be grown in the laboratory setting in the past, but only with limited success. It was believed that urothelial cells had a natural senescence that was hard to overcome. Several protocols have been developed over the last two decades that have improved urothelial growth and expansion.\(^7^1–^7^4\) A system of urothelial cell harvesting was developed that does not use any enzymes or serum and has a large expansion potential. Using these methods of cell culture, it is possible to expand a urothelial strain from a single specimen that initially covers a surface area of 1 cm\(^2\) to one covering a surface area of 4202 m\(^2\) (the equivalent area of one football field) within 8 weeks.\(^7^1\)

An advantage of native targeted progenitor cells is that they are already programmed to become the cell type needed, and no *in vitro* differentiation steps are required for their use in the organ of origin. An additional advantage in using native cells is that they can be
Bladder, ureter and renal pelvis cells can all be harvested, cultured and expanded in a similar fashion. Normal human bladder epithelial and muscle cells can be efficiently harvested from surgical material, extensively expanded in culture, and their differentiation characteristics, growth requirements and other biologic properties can be studied. Major advances in cell culture techniques have been made within the past decade, and these techniques make the use of autologous cells possible for clinical application.

Another major concern has been that, in cases where cells must be expanded from a diseased organ, there may no longer be enough normal cells present in that organ to begin the process. Recent research suggests that this may not be the case, however. For example, one study has shown that cultured neuropathic bladder smooth muscle cells possess and maintain different characteristics than normal smooth muscle cells in vitro, as demonstrated by growth assays, contractility and adherence tests in vitro. Despite these differences, when neuropathic smooth muscle cells were cultured in vitro, and then seeded onto matrices and implanted in vivo, the tissue-engineered constructs showed the same properties as the constructs engineered with normal cells. It is now known that genetically normal progenitor cells, which are the reservoirs for new cell formation, are present even in diseased tissue. These normal progenitors are programmed to give rise to normal tissue, regardless of whether they reside in a normal or diseased environment. Therefore, the stem cell niche and its role in normal tissue regeneration remains a fertile area of ongoing investigation.

**Stem cells and other pluripotent cell types**

As discussed, most current strategies for tissue engineering depend upon a sample of autologous cells from the diseased organ of the host. In some instances, primary autologous human cells cannot be expanded from a particular organ, such as the pancreas, or there is not enough normal tissue remaining in the diseased organ to use for the procedures described above. In these situations, pluripotent human stem cells are envisioned to be an ideal source of cells, as they can differentiate into nearly any replacement tissue in the body.

Embryonic stem (ES) cells exhibit two remarkable properties: the ability to proliferate in an undifferentiated, but still pluripotent state (self-renewal) and the ability to differentiate into a large number of specialized cell types. They can be isolated from the inner cell mass of the embryo during the blastocyst stage, which occurs 5 days post-fertilization. These cells have been maintained in the undifferentiated
state for at least 80 passages when grown using current published protocols. In addition, many protocols for differentiation into specific cell types in culture have been published. ES cells tend to form teratomas when implanted in vivo due to their pluripotent state, and the cells are not autologous, so may present problems with rejection, limiting their clinical application at this time.

Adult stem cells, especially hematopoietic stem cells, are the best understood cell type in stem cell biology. Despite this, adult stem cell research remains an area of intense study, as their potential for therapy may be applicable to a myriad of degenerative disorders. Within the past decade, adult stem cell populations have been found in many adult tissues other than the bone marrow and the gastrointestinal tract, including the brain, skin and muscle. Many other types of adult stem cells have been identified in organs all over the body and are thought to serve as the primary repair entities for their corresponding organs. The discovery of such tissue-specific progenitors has opened up new avenues for research.

A notable exception to the tissue specificity of adult stem cells is the mesenchymal stem cell, also known as the multipotent adult progenitor cell. This cell type is derived from bone marrow stroma. Such cells can differentiate in vitro into numerous tissue types and can also differentiate developmentally if injected into a blastocyst. Multipotent adult progenitor cells can develop into a variety of tissues including neuronal, adipose, muscle, liver, lungs, spleen and gut tissue, but notably not bone marrow or gonads.

Research into adult stem cells has, however, progressed slowly, mainly because investigators have had difficulty in maintaining adult non-mesenchymal stem cells in culture. Some cells, such as those of the liver, pancreas and nerve, have very low proliferative capacity in vitro, and the functionality of some cell types is reduced after the cells are cultivated. Isolation of cells has also been problematic, because stem cells are present in extremely low numbers in adult tissue. While the clinical utility of adult stem cells is currently limited, great potential exists for future use of such cells in tissue-specific regenerative therapies. The advantage of adult stem cells is that they can be used in autologous therapies, thus avoiding any complications associated with immune rejection.

The isolation of multipotent human and mouse amniotic-fluid and placental-derived stem (AFPS) cells that are capable of extensive self-renewal and give rise to cells from all three germ layers was reported in 2007. AFPS cells represent ~1% of the cells found in the amniotic fluid and placenta. The undifferentiated stem cells expand extensively without a feeder cell layer and double every 36 h. Unlike human ES cells, the AFPS cells do not form tumors in vivo. Lines maintained for
over 250 population doublings retained long telomeres and a normal complement of chromosomes. AFPS cell lines can be induced to differentiate into cells representing each embryonic germ layer, including cells of adipogenic, osteogenic, myogenic, endothelial, neural-like and hepatic lineages. In addition to the differentiated AFPS cells expressing lineage-specific markers, such cells can have specialized functions. Cells of the hepatic lineage secreted urea and α-fetoprotein, while osteogenic cells produced mineralized calcium. In this respect, they meet a commonly accepted criterion for multipotent stem cells, without implying that they can generate every adult tissue.

AFS cells represent a new class of stem cells with properties somewhere between those of ES and adult stem cell types, probably more agile than adult stem cells, but less so than ES cells. Unlike embryonic and induced pluripotent stem cells, however, AFPS cells do not form teratomas, and if preserved for self-use, avoid the problems of rejection. The cells could be obtained either from amniocentesis or chorionic villous sampling in the developing fetus, or from the placenta at the time of birth. They could be preserved for self-use, and used without rejection, or they could be banked. A bank of 100 000 specimens could potentially supply 99% of the US population with a perfect genetic match for transplantation. Such a bank may be easier to create than with other cell sources, since there are ~4.5 million births per year in the USA.

Since the discovery of the AFPS cells, other groups have published on the potential of the cells to differentiate to other lineages, such as cartilage,\(^{117}\) kidney,\(^{118}\) and lung.\(^{119}\) Muscle-differentiated AFPS cells were also noted to prevent compensatory bladder hypertrophy in a cryo-injured rodent bladder model.\(^{120}\)

Nuclear transfer, or cloning, can serve as another source of pluripotent ‘stem’ cells that could possibly be used for regenerative medicine therapies. Two types of cloning procedures exist—reproductive cloning and therapeutic cloning. Banned in most countries for human applications, reproductive cloning is used to generate an embryo that has identical genetic material to its cell source. This embryo is then implanted into the uterus of a pseudopregnant female to give rise to an infant that is a clone of the donor. While therapeutic cloning also produces an embryo that is genetically identical to the donor nucleus, this process is used to generate blastocysts that are explanted and grown in culture, rather than in utero, to produce ES cell lines. These autologous stem cells have the potential to become almost any type of cell in the adult body, and thus would be useful in tissue and organ replacement applications.\(^{121}\) Therefore, therapeutic cloning, which has also been called somatic cell nuclear transfer, may provide an alternative source of transplantable cells that are identical to the patient’s own cells.
More recently, successful transformation of adult cells into pluripotent stem cells through genetic ‘reprogramming’ has been possible. Reprogramming is a technique that involves de-differentiation of adult somatic cells to produce patient-specific pluripotent stem cells, without the use of embryos. Cells generated by reprogramming would be genetically identical to the somatic cells (and thus, the patient who donated these cells) and would not be rejected. Yamanaka\textsuperscript{122} was the first to discover that mouse embryonic fibroblasts and adult mouse fibroblasts could be reprogrammed into an ‘induced pluripotent state (iPS)’. They examined 24 genes that were thought to be important for ES cells and identified 4 key genes that were required to bestow ES cell-like properties on fibroblasts—Oct3/4, Sox2, c-Myc and Klf4. iPS cells in this study possessed the immortal growth characteristics of self-renewing ES cells, expressed genes specific for ES cells and generated embryoid bodies \textit{in vitro} and teratomas \textit{in vivo}. When iPS cells were injected into mouse blastocysts, they contributed to a variety of cell types in the embryo. However, although iPS cells selected in this way were pluripotent, they were not identical to ES cells. Unlike ES cells, chimeras made from iPS cells did not result in full-term pregnancies. Gene expression profiles of the iPS cells showed that they possessed a distinct gene expression signature that was different from that of ES cells. In addition, the epigenetic state of the iPS cells was somewhere between that found in somatic cells and that found in ES cells, suggesting that the reprogramming was incomplete.

These results were improved significantly by Wernig and Jaenisch\textsuperscript{123} in July 2007. Results from this study showed that DNA methylation, gene expression profiles and the chromatin state of the reprogrammed cells were similar to those of ES cells. Teratomas induced by these cells contained differentiated cell types representing all three embryonic germ layers. Most importantly, the reprogrammed cells from this experiment were able to form viable chimeras and contribute to the germ line like ES cells, suggesting that these iPS cells were completely reprogrammed.

It has recently been shown that reprogramming of human cells is possible.\textsuperscript{124,125} Yamanaka showed that retrovirus-mediated transfection of OCT3/4, SOX2, KLF4 and c-MYC generates human iPS cells that are similar to hES cells in terms of morphology, proliferation, gene expression, surface markers and teratoma formation. Thompson’s group showed that retroviral transduction of OCT4, SOX2, NANOG and LIN28 could generate pluripotent stem cells without introducing any oncogenes (c-MYC). Both studies showed that human iPS were similar but not identical to hES cells. However, iPS cells, like HES, have a tendency to form tumors (teratomas).
Tissue engineering strategies for bladder replacement

Biomaterial matrices for bladder regeneration

Over the last few decades, several bladder wall substitutes have been attempted with both synthetic and organic materials. Synthetic materials that have been tried in experimental and clinical settings include polyvinyl sponges, Teflon, collagen matrices, Vicryl (PGA) matrices and silicone. Most of these attempts have failed because of mechanical, structural, functional or biocompatibility problems. Usually, permanent synthetic materials used for bladder reconstruction succumb to mechanical failure and urinary stone formation, and use of degradable materials leads to fibroblast deposition, scarring, graft contracture, and a reduced reservoir volume over time.78,126

There has been a resurgence in the use of various collagen-based matrices for tissue regeneration. Non-seeded allogeneic acellular bladder matrices have served as scaffolds for the ingrowth of host bladder wall components. The matrices are prepared by mechanically and chemically removing all cellular components from bladder tissue.51,52,54,127,128 The matrices serve as vehicles for partial bladder regeneration and relevant antigenicity is not evident.

Cell-seeded allogeneic acellular bladder matrices have been used for bladder augmentation in dogs.52 The regenerated bladder tissues contained a normal cellular organization consisting of urothelium and smooth muscle and exhibited a normal compliance. Biomaterials preloaded with cells before their implantation showed better tissue regeneration compared with biomaterials implanted with no cells, in which tissue regeneration depended on ingrowth of the surrounding tissue. The bladders showed a significant increase (100%) in capacity when augmented with scaffolds seeded with cells, compared with scaffolds without cells (30%). The acellular collagen matrices can be enhanced with growth factors to improve bladder regeneration.129

SIS, a biodegradable, acellular, xenogeneic collagen-based tissue-matrix graft, was first described by Badylak et al.130 in the 1980s as an acellular matrix for tissue replacement in the vascular field. It has been shown to promote regeneration of a variety of host tissues, including blood vessels and ligaments.131 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 the non-seeded SIS matrix used for bladder augmentation is able to regenerate in vivo.132,133 Histologically, the transitional layer was the same as that of the native bladder tissue, but, as with other non-seeded collagen matrices used experimentally, the muscle layer was not fully developed. A large
amount of collagen was interspersed among a smaller number of muscle bundles. A computer-assisted image analysis demonstrated a decreased muscle-to-collagen ratio with loss of the normal architecture in the SIS-regenerated bladders. In vitro contractility studies performed on the SIS-regenerated dog bladders showed a decrease in maximal contractile response by 50% from those of normal bladder tissues. Expression of muscarinic, purinergic and alpha-adrenergic receptors and functional cholinergic and purinergic innervation was demonstrated.\textsuperscript{133} Cholinergic and purinergic innervation also occurred in rats.\textsuperscript{134}

Bladder augmentation using laparoscopic techniques was performed on minipigs with porcine bowel acellular tissue matrix, human placental membranes or porcine SIS. At 12 weeks post-operatively, the grafts had contracted to 70, 65 and 60\% of their original sizes, respectively, and histologically the grafts showed predominantly only mucosal regeneration.\textsuperscript{135} The same group evaluated the long-term results of laparoscopic hemicystectomy and bladder replacement with SIS with ureteral reimplantation into the SIS material in minipigs. Histopathology studies after 1 year showed muscle at the graft periphery and center but it consisted of small fused bundles with significant fibrosis. Nerves were present at the graft periphery and center but they were decreased in number. Compared to primary bladder closure after hemi-cystectomy, no advantage in bladder capacity or compliance was documented.\textsuperscript{136} More recently, bladder regeneration has been shown to be more reliable when the SIS was derived from the distal ileum.\textsuperscript{137}

In multiple studies using various materials as non-seeded grafts for cystoplasty, the urothelial layer was able to regenerate normally, but the muscle layer, although present, was not fully developed.\textsuperscript{52,54,127,133,138,139} Studies involving acellular matrices that may provide the necessary environment to promote cell migration, growth and differentiation are being conducted.\textsuperscript{140} With continued bladder research in this area, these matrices may have a clinical role in bladder replacement in the future.

**Regenerative medicine for bladder using cell transplantation**

Regenerative medicine with selective cell transplantation may provide a means to create functional new bladder segments.\textsuperscript{77} The success of cell transplantation strategies for bladder reconstruction depends on the ability to use donor tissue efficiently and to provide the right conditions for long-term survival, differentiation and growth. Various cell sources have been explored for bladder regeneration. Native cells are currently preferable due to their autologous source, wherein they can
be used without rejection. It has been shown experimentally that the bladder neck and trigone area has a higher propensity of urothelial progenitor cells, and these cells are localized in the basal region. 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. ES cells also have the potential to differentiate into bladder tissue.

Human urothelial and muscle cells can be expanded in vitro, seeded onto polymer scaffolds and allowed to attach and form sheets of cells. The cell-polymer scaffold can then be implanted in vivo. Histologic analysis indicated that viable cells were able to self-assemble back into their respective tissue types, and would retain their native phenotype. These experiments demonstrated, for the first time, that composite layered tissue-engineered structures could be created de novo. Before this study, only non-layered structures had been created in the field of regenerative medicine.

It has been well established for decades that portions of the bladder are able to regenerate generously over free grafts, most likely because the urothelium is associated with a high reparative capacity. However, bladder muscle tissue is less likely to regenerate in a normal fashion. Both urothelial and muscle ingrowth are believed to be initiated at the edges of the injury, from the normal bladder tissue in towards the region of the free graft. Usually, however, contraction or resorption of the graft has been evident. Inflammation in response to the matrix may contribute to the resorption of the free graft. As a result of this discovery, it was hypothesized that building the three-dimensional bladder constructs in vitro, before implantation, would facilitate the eventual terminal differentiation of the cells after implantation in vivo and would minimize the inflammatory response toward the matrix, thus avoiding graft contracture and shrinkage. The dog study described earlier supports this hypothesis and illustrates a major difference between matrices used with autologous cells (tissue-engineered matrices) and those used without cells. Matrices that were seeded with cells and then used for bladder augmentation retained most of their preimplantation diameter, as opposed to matrices implanted without cells, in which significant graft contraction and shrinkage occurred. In addition, histological analysis demonstrated a marked paucity of muscle cells and a more aggressive inflammatory reaction in the matrices implanted without cells.

The results of these initial studies showed that the creation of artificial bladders may be achieved in vivo; however, it could not be determined whether the functional parameters noted were created by the augmented segment or by the remaining native bladder tissue. To better address this question, an animal model was designed in which
subtotal cystectomies followed by replacement with a tissue-engineered organ were performed.\textsuperscript{85} Cystectomy-only controls and animals that received bladder replacements made from non-seeded matrices maintained average capacities of 22 and 46\% of preoperative values, respectively. However, an average bladder capacity of 95\% of the original precystectomy volume was achieved in animals receiving cell-seeded tissue engineered bladder replacements. These findings were confirmed radiographically. The subtotal cystectomy reservoirs that were not reconstructed and the polymer-only reconstructed bladders showed a marked decrease in bladder compliance (10 and 42\% total compliance). In contrast, the compliance of the cell-seeded tissue-engineered bladders showed almost no difference from preoperative values that were measured when the native bladder was present (106\%). Histologically, the non-seeded bladder replacement scaffolds presented a pattern of normal urothelial cells with a thickened fibrotic submucosa and a thin layer of muscle fibers. The tissue-engineered bladders (scaffold + cells) showed a normal cellular organization, consisting of a trilayer of urothelium, submucosa and muscle. Immunocytochemical analyses confirmed the muscle and urothelial phenotype. S-100 staining indicated the presence of neural structures.\textsuperscript{85} These studies have been repeated by other investigators, and they obtained similar results using larger numbers of animals over the long term.\textsuperscript{138,149} Thus, the strategy of using biodegradable scaffolds seeded with cells can be pursued without concerns for local or systemic toxicity.\textsuperscript{150}

However, not all scaffold materials perform well if a large portion of the bladder must be replaced. 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.\textsuperscript{151} This confirms that the type of scaffold used in the construction of tissue-engineered bladders is critical for the success of these technologies. The use of bioreactors, which provide mechanical stimulation for the growing organ \textit{in vitro}, has also been proposed as an important parameter for success.\textsuperscript{152} Bioreactors provide can provide mechanical stimulation such as periodic stretching of the tissue, which has been shown to assist in \textit{in vitro} muscle development, and exposure to flow conditions, which is important for the development of endothelial layers in blood vessels and hollow organs such as the bladder. In fact, Farhat and Yeger\textsuperscript{152} have developed bioreactor systems specifically for bladder development. These systems provide simulated filling/emptying functions to the engineered tissue, and this may lead to a bladder construct with more functionality.

A clinical experience involving engineered bladder tissue for cystoplasty was conducted starting in 1998. A small pilot study of seven
patients reported the use of either collagen scaffolds seeded with cells or a combined PGA-collagen scaffold seeded with cells for bladder replacement. These engineered tissues were implanted with or without omental coverage (Fig. 1). Patients reconstructed with engineered bladder tissue created with cell-seeded PGA-collagen scaffolds and omental coverage showed increased compliance, decreased end-filling pressures, increased capacities and longer dry periods over time (Fig. 2). It is clear from this experience that the engineered bladders continued to improve with time, mirroring their continued development.

In repeat clinical trials at other centers, patients were observed to repeat the published findings to the first year post-implantation (Joseph et al., presented at the Annual Meeting of the American Urological Association, 2008). Patients who responded to the regenerative medical

Fig. 1 Construction of engineered bladder. (A) Scaffold material seeded with cells for use in bladder repair. (B) The seeded scaffold is anastomosed to native bladder with running 4-0 polyglycolic sutures. (C) Implant covered with fibrin glue and omentum.

Fig. 2 Cystograms and urodynamic studies of a patient before and after implantation of the tissue engineered bladder. (A) Preoperative results indicate an irregular-shaped bladder in the cystogram (left) and abnormal bladder pressures as the bladder is filled during urodynamic studies (right). (B) Postoperatively, findings are significantly improved.
product regenerated bladder tissue, increased capacity and had reduction of intravesiocular pressures when compared with the baseline. Based on insights from these clinical trials, an autologous regenerative medical product may have clinical utility for neurogenic bladder while avoiding many of the complications associated with using bowel tissue in urologic procedures.

Although the experience to date is promising and shows that engineered tissues can be implanted safely, it is just a first step towards the goal of engineering fully functional bladders. Only a limited clinical experience exists to date, and the technology is not yet ready for wide dissemination, as further experimental and clinical studies are required.

In the past, an important area of concern in tissue engineering was the quality of the source of cells for regeneration. The concept of creating engineered constructs by obtaining cells for expansion from the diseased organ led investigators to consider whether or not the cell population derived and expanded from diseased tissue would be normal, with normal functional parameters. For example, would the cells obtained from a neuropathic bladder lead to the formation of normal bladder tissue or to the engineering of another neuropathic bladder? It has been shown that cultured neuropathic bladder smooth muscle cells possess different characteristics than normal smooth muscle cells in vitro, as demonstrated by growth assays, contractility, adherence tests and microarray analysis. However, when neuropathic smooth muscle cells were cultured in vitro, and seeded onto matrices and implanted in vivo, the tissue-engineered constructs showed the same properties as the tissues engineered with normal cells. Thus, it appears that genetically normal non-malignant progenitor cells are programmed to give rise to normal tissue, regardless of whether they exist in normal or diseased tissues Therefore, although the mechanisms for tissue self-assembly and regenerative medicine are not fully understood, it is known that the progenitor cells are able to ‘reset’ their program for normal cell differentiation. The stem cell niche and its role in normal tissue regeneration remains a fertile area of ongoing investigation.

Conclusions

From the above studies, it is evident that the use of cell-seeded matrices is superior to the use of non-seeded matrices for the creation of engineered bladder tissues. Although advances have been made with the engineering of bladder tissues, many challenges remain. Current research in many centers is aimed at the development of biologically
active and ‘smart’ biomaterials that may improve bladder tissue regeneration as well as regeneration of many other tissues in the body.

Acknowledgements

The author would like to thank Dr. Jennifer Olson for editorial assistance with this manuscript.

Conflict of interest: Dr. Atala is a consultant/advisor to Tengion, Inc. (Winston-Salem, NC).

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