

# Bladder reconstruction: The past, present and future (Review)

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**Abstract.** Ileal conduit urinary diversion is the gold standard treatment for urinary tract reconstruction following cystectomy. This procedure uses gastrointestinal segments for bladder augmentation, a technique that is often associated with significant complications. The substantial progression in the fields of tissue engineering and regenerative medicine over the previous two decades has resulted in the development of techniques that may lead to the construction of functional *de novo* urinary bladder substitutes. The present review identifies and discusses the complications associated with current treatment options post-cystectomy. The current techniques, achievements and perspectives of the use of biomaterials and stem cells in the field of urinary bladder reconstruction are also reviewed.

## Contents

1. Introduction
2. Biomaterials in bladder regeneration
3. The use of unseeded and cell-seeded matrices in bladder regeneration
4. Stem cells in bladder regeneration
5. Conclusion and perspectives

## 1. Introduction

*The past and present.* The urinary bladder is an elastic reservoir that is responsible for the low pressure storage of urine. A continent, pain free and healthy bladder is crucial for the preservation of a good quality of life. Numerous conditions

disturb the anatomy and physiology of the urinary bladder, leading to insufficient and restricted evacuation of urine (1).

Bladder cancer (BC) is the sixth most common cause of cancer-associated morbidity in the western world: In the UK in 2010, there were 10,300 new cases, and 4,900 cases of BC-associated mortality (2). It represents the most common malignancy of the urinary tract, with a median survival rate following metastasis that rarely surpasses 15 months (3). The vast majority (90%) of bladder tumours are histologically classified as urothelial cell carcinoma, these tumours are often further classified as being non-muscle invasive BC (NMIBC) or muscle invasive BC (MIBC) (4). On initial presentation, 30% of patients are diagnosed with an MIBC tumour. From the remainder of patients that had been diagnosed with an NMIBC tumour at presentation, 30% go on to develop an MIBC during follow-up (5). With the highest susceptibility of recurrence and progression of any malignancy, patients with BC require consistent surveillance and close follow-up during and, more importantly, following therapy. With such high odds of recurrence, BC has produced the most expensive management protocol of any malignancy, costing the National Health Service ~£55.39 million in the UK per annum (6). The gold standard treatment for organ-confined MIBC is radical cystectomy, with dissection of the pelvic lymph nodes. Regardless, malignancy is not the sole bladder-associated pathology in which cystectomy is indicated (7).

Bladder damage occurs in several additional disorders within the genitourinary (GU) system, including inflammatory conditions (interstitial cystitis/bladder pain syndrome), urinary tract infections, nerve damage (neuropathic bladder) and congenital disorders (spina bifida). In these cases, the essential task is to re-establish the function of the urinary bladder. Medical treatments are limited, and thus, surgical intervention, particularly cystectomy, is often required (8-11).

Since the first BC-associated cystectomy in 1887, the pursuit to identify the most appropriate replacement voiding system has been largely problematic (12). At present, the most common method of bladder replacement or repair involves the use of autologous segments of gastrointestinal tissue, in order to restore bladder storage and voiding capacity (13). This is often achieved through ileal conduit urinary diversion, orthotopic bladder substitution or continent cutaneous diversion (Table I) (14,15).

Since the original application of a free tissue graft in 1917, in which canine bladders were augmented with fascia, various

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Table I. Surgical procedures used post cystectomy.

Technique	Description
Ileal conduit urinary diversion	Urine is drained from the ureters to a loop of small bowel anastomosed to the abdominal skin surface. It is then collected in an external appliance.
Orthotopic bladder substitution	This practice imitates the typical role of the urinary bladder through using a section of bowel to reconstruct the bladder.
Continent cutaneous diversion	This method uses the ileocaecal valve which may aid the regulation of urination. This procedure utilises the bowel, which is deployed to mimic the urinary bladder. The ureters are attached to the pouch. The pouch is then brought to the skin as a stoma.
Ureterosigmoidostomy	Possibly the oldest procedure, here the ureters are attached to the large bowel and urine exits this way. The anal sphincter provides continence.

Table II. Complications of current bladder augmentation procedures through the use of gastrointestinal tissue in the urinary tract.

Complication	Description
Electrolyte disturbances	Hyperchloremic, hypokalaemic metabolic acidosis is the most common complication. A number of these patients will be required to take life long oral Sodium bicarbonate as a result.
Vitamin deficiency	Loss of segments of bowel or stomach can impair vitamin B12/ bile salt/fat/fat soluble vitamin absorption. Vitamin B12 deficiency can manifest as peripheral neuropathy.
Drug metabolism	Drugs can be reabsorbed by the intestinal segments that have been incorporated into the urinary tract, causing toxicity. Methotrexate, phenytoin and lithium in particular result in therapeutic changes. Dose adaptation and close surveillance is often required.
Hepatic metabolism	The intestinal segments that are used for diversion will drain into the portal circulation and consequently, into the liver. This abnormal portal system communication can lead to ammonia toxicity, manifesting as ammoniagenic encephalopathy.
Bone disease	This is generally a long term complication of diversion which has been demonstrated in adults with osteomalacia and children with rickets. The pathological process is complex, but it has been attributed to chronic acidosis.
Cancer	Tumours have been reported close to the anastomotic sites and include transitional cell carcinoma, adenocarcinoma, sarcoma and adenomatous polyps.
Surgical	These are numerous and can manifest early or late. The most common complications are stomal, including stomal bleeds, parastomal hernias and stomal stenosis.

additional materials have been utilized as free grafts (16). The ileum, however, is the most frequently utilized segment in bladder augmentation, due to its notably simple mobilization, extensive mesentery and copiousness; features that are particularly appropriate for urinary tract substitution (17). Although the use of gastrointestinal segments in bladder reconstruction was first proposed almost 150 years ago, it remains the gold standard due to the absence of a superior alternative. This was demonstrated by Stein *et al* (18), who observed excellent long-term survival rates in patients undergoing radical cystectomy. Whilst radical extirpation of the bladder is frequently successful, from an oncological perspective, substantial morbidity is associated with enteric interposition within the GU tract (Table II) (19-22). Surgical intervention itself requires procedures on the urinary and gastrointestinal tracts which are associated with complication rates of  $\leq 66\%$ . Considering that the vast majority of patients that require cystectomy and consequent bladder substitution are elderly and suffer from other co-morbidities, in particular

renal impairment, urologists find themselves fighting an uphill battle in surgical management post-cystectomy (16).

Certain bladder pathologies, in particular metastatic BC, are managed medically prior to considering radical cystectomy. This method of organ preservation involves aggressive treatment through surgery and radiotherapy, often with neo-adjuvant chemotherapy as the treatment of choice (23). Currently, only multidrug platinum-based chemotherapeutic regimens have been successful (24,25), whereas monotherapy treatments have failed (26,27). The methotrexate, vinblastine, adriamycin and cisplatin (MVAC) and gemcitabine and cisplatin (gem-cis) regimens have been the most commonly used chemotherapeutic treatment regimens. However, the clinical efficacy of cisplatin-based therapy in BC is currently restricted by the rapid development of drug resistance in the majority of patients, frequently leading to therapeutic failure (28). This was demonstrated in a previous study in which response rates from BC patients receiving chemotherapeutic drug intervention in phase II trials varied between 20 and 60%, with the

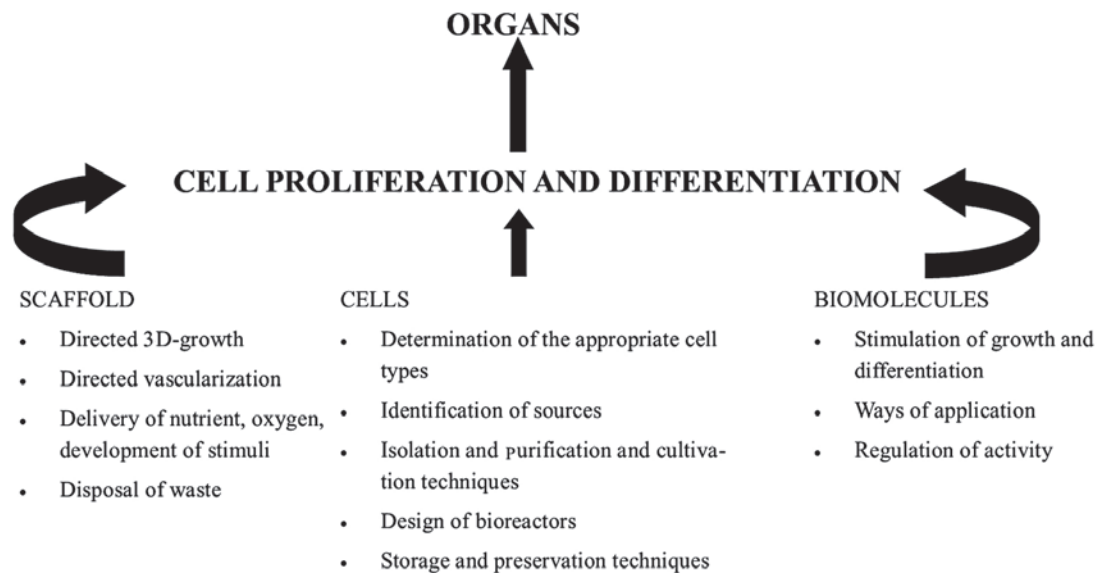


Figure 1. Constituents of tissue engineering. The idea of bladder reconstruction is to design and ultimately construct the perfect artificial bladder. This is achieved through identifying vital pathways for tissue regeneration and encouraging these pathways by providing suitable progenitor cells, a scaffold of suitable quality to direct growth, an adequate blood supply and adequate cell signalling regulation.

majority of survival rate advantage in the randomised control trial setting (29,30). This has resulted in the ever apparent requirement for bladder reconstruction.

*The future.* The absence of autologous tissue with parallel properties to the native bladder has directed numerous studies to develop alternative methods of bladder substitution, avoiding the use of bowel tissue altogether. The theory of substituting the native bladder with a synthetic prosthesis has always been an attractive prospect in the development of cutting edge technology. Thus, tissue engineering, cell and stem cell biology, material science and regenerative medicine are at the forefront of medical research (31).

The fields of tissue engineering and regenerative medicine have witnessed substantial progression over the previous two decades. Various studies have investigated the possibility of regenerating multilayer urothelium, which led to the first clinical trial in 2006, in which Atala *et al* (32) investigated tissue engineered bladders created from cell seeded grafts. The potential of such novel findings has underlined the requirement for further advances in tissue engineering and material science in order to define the properties required for the ultimate reconstructive material and method of implantation.

Tissue engineering is the mainstay of regenerative medicine. It employs the disciplines of cell biology, transplantation, material science and biomedical engineering, towards identifying alternatives that can re-establish and preserve the regular function of damaged tissues and organs (Fig. 1) (33). Although the human body is outstanding in its ability to repair damaged tissue, these reparative processes are frequently restricted to the development of scar tissue. This often proves detrimental in the function of the bladder (34). The ideal artificial bladder should possess properties similar to that of the native urinary bladder. It should possess the ability to store urine at low pressure in a watertight structure, similar to a mechanical reservoir, and permit voluntary voiding with minimal reflux.

This structure should also be constructed from inert material and cause minimal complications in the patient so that long-term renal function is not compromised (35). Previously published animal studies have demonstrated promising results in the field of regenerative medicine, and it represents a possible solution for the treatment of a number of urological conditions in the future (31).

Tissue engineering strategies vary, and currently, studies are being orientated in two directions: Firstly, to identify the most appropriate type of stem cell for regeneration and to proficiently incorporate it into bladder cells; secondly, to determine the most appropriate material and technique of embedding these cells using tissue engineered grafts (Fig. 2) (36,37). The selected grafts must exhibit all the qualities of the native tissue, acting ultimately as microenvironments for the implanted cells to prosper (38).

## 2. Biomaterials in bladder regeneration

There are distinct benefits to using biocompatible material in regenerative medicine for the purpose of cell delivery vehicles, and for bearing the physical maintenance required for tissue replacement (39). Scaffolds are constructs that are designed to direct tissue development and the growth of cells during the process of healing (40). Bladder replacements should therefore provide provisional mechanical support, adequate to endure forces exerted from neighbouring structures, whilst maintaining a potential zone for tissue development. Biomaterials used for bladder replacements should possess the ability to be easily manipulated into a hollow, spherical configuration. Furthermore, the biomaterials should possess the ability to biodegrade for complete tissue development, without causing inflammation. Autologous tissue has been experimented on for bladder repair since the early 1980s (41). The use of omentum, pericardium, stomach and skin has been attempted with limited success (42-45). It was the lack of watertight properties

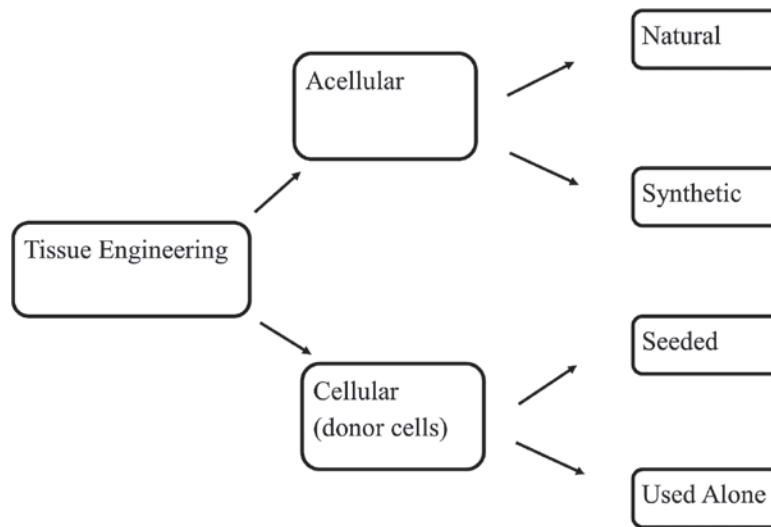


Figure 2. Tissue engineering strategies. Tissue engineering approaches can be acellular or cellular. The acellular strategy uses natural or synthetic scaffolds that aim to improve the body's own capability at repairing itself and differentiating into new tissue. The cellular strategy uses donor cells that can be seeded by incorporation into the scaffold or used alone (stem cell approach). At present, the cell seeded approach has demonstrated superiority in the formation of a tissue engineered bladder.

that led to the failure of these materials. It is clear that the anatomical and physiological properties of the urinary bladder are not easily substituted.

Biomaterials can be divided into 3 main categories: i) Naturally derived matrices, including collagen; ii) acellular tissue matrices, including bladder submucosa; and iii) synthetic matrices, including poly lactic-co-glycolic acid (PLGA) (46).

*Naturally derived matrices.* Collagen is considered to be the most ubiquitous protein in the human body, and it is often used alongside alginate as a natural matrix. It is useful in tissue engineering, as it possesses the ability to be easily manipulated and does not provoke an immune response (47). Through the use of innovative inkjet technology, it has been possible to use bio-printing to create a naturally derived 3D construct, with a precise arrangement of growth factors and other cellular components, into a patient-specific scaffold (48).

*Acellular tissue matrices.* Decellularised matrices are the most commonly used naturally derived urological matrices. They are usually harvested from autologous, allogenic or xenogenic tissue (49,50). Chemical or mechanical processing decellularises the matrix, removing all cellular components and leaving a natural platform for tissue development (51). The most common origin of decellularised matrices is tissue harvested from the bladder or small intestinal mucosa (52). Once implanted, the matrices eventually degrade and are ultimately replaced by an extracellular matrix. The matrices provide cells with a structural support that can dictate the tissue structure. Bladder acellular matrix may be the most extensively used scaffold in tissue engineering. It has been demonstrated that the cells have the ability to induce the ingrowth of urothelium, smooth muscle, endothelium and nerve cells (53). Perhaps the greatest advantage of the use of acellular matrices is the ability to provide a method of neovascularisation following graft insertion, promoting graft survival. Kikuno *et al* (54) demonstrated that nerve growth factor and vascular endothelial

growth factor enhanced bladder acellular matrix grafting in neurogenic rat bladders through increased angiogenesis and neurogenesis (55).

However, in general, these materials possess various drawbacks depending on their graft origin. Autologous materials have proven difficult to reproduce as a result of increased patient morbidity associated with the graft harvest (56). The increased cost in allograft and xenograft material production, in addition to the risk of disease transmission and varied mechanical strength, is crucial in their limited clinical application (56,57). Furthermore, the use of natural decellularised matrices requires tissue that exhibits no principal pathological change, and is therefore unfeasible in certain patients (58,59). In addition, the aggressive decellularisation and sterilisation protocols used can denature proteins in the extracellular matrix, damaging the physiological environment (60).

*Synthetic matrices.* The use of synthetic material in patients in which the native bladder has undergone pathological change, such as BC, is hypothesised to be the ideal solution. The most commonly used materials in experimental studies and clinical trials are Teflon, silicon and collagen matrices (61). However, cell and tissue incompatibilities appear to be the major restrictions of these materials, leading to the development of other synthetic polymers, such as PLGA (62-65). These materials were designed specifically to possess adequate structural and biological properties, which can be manipulated for optimal cell proliferation and differentiation (66). In addition, these scaffolds are safe, possess controlled properties of degradation rate and strength, are readily available and possess the ability to carry vital growth factors, such as vascular endothelial growth factor, for neovascularisation (67-69). Indeed, the most attractive property of PLGA compared with older polymers, such as Teflon, is its resorbable biodegradability. This eliminates the disadvantages of infection, calcification and unfavourable connective tissue responses observed in older polymers (70). When using polymers, tissue recognition is difficult, and often



requires immunosuppressive therapy. However, it has now been demonstrated that novel biological factors, including adhesive proteins such as collagen and fibronectin and growth factors such as basic fibroblast growth factor, epidermal growth factor and insulin, are able to enhance cell recognition (71,72).

The failure of certain materials in clinical cases has been extensively reported, including plastic moulds (73,74), gelatin sponges (75,76), Japanese paper produced from the rice paper plant (*Tetrapanax papyrifer*) (77,78) and bovine pericardium (79,80).

Nanotechnology, which arose in the last decade of the 20th century, has been used in the formation of matrices that can be composed of natural and synthetic materials. The use of nanotechnology permits the development of a graft that can be manipulated to produce characteristics with definitive properties in order to promote optimal cell proliferation and tissue differentiation (53). A previous study investigated the effect of surface roughness on the interaction with matrix proteins (81). Vitronectin absorbance improved by 20% with the addition of nanotechnology-induced surface roughness in the synthetic materials compared with the nanosmooth surface. Similarly, enhanced adhesion and proliferation of urothelial cells has been demonstrated with increased synthetic surface roughness (82).

### 3. The use of unseeded and cell-seeded matrices in bladder regeneration

Although there have been studies illustrating the use of unseeded matrices in bladder tissue engineering, the results of these studies have been inconclusive. The normal regeneration of the urothelium layer has been demonstrated in unseeded grafts used for cystoplasty, however, the muscular layer did not develop (83). By contrast, the cell-seeded approach has produced more positive and consistent results in bladder reconstruction, and has been the most extensively investigated strategy in reconstruction. This approach involves seeding of a scaffold with autologous patient-derived cells, which is then transplanted back into the patient to accomplish regeneration (32).

In a study conducted on beagle dogs following subtotal cystectomy, polyglycolic acid acellular matrices were seeded with urothelium and smooth muscle cells. These dogs demonstrated a 95% increase in pre-operative bladder capacity compared with dogs treated with an unseeded matrix, indicating a 46% increase in pre-operative bladder capacity (84).

The study by Atala *et al* (32) was crucial for the first laboratory-created organ to be transplanted into the human body. The authors demonstrated an increase in bladder compliance and capacity, and a reduction in end filling pressure in 7 patients with neurogenic bladder (myelomeningocele). These patients underwent cystoplasty created from autologous cells seeded on collagen-polyglycolic acid scaffolds. This small clinical study established the possibility of using tissue-engineered substitutes for organ replacements in humans, circumventing the complications of intestinal substitutes (32).

### 4. Stem cells in bladder regeneration

Historically, tissue engineering has depended on autologous cells from the host organ. However, physiologically normal tissue may not always be available for harvest to elaborate a

regenerative cystoplasty, this has led to the use of stem cells as an alternative to restore urinary bladder function. The aim of using stem cells as a therapeutic option is to achieve adequate differentiation into urothelial cells and smooth muscle cells, so that the normal histological structure is maintained for use as a potential resource for cell-based therapy in urology (85).

Embryonic stem cells are a group of pluripotent stem cells and exhibit two valuable attributes, including the capability to undergo self-renewal and the capability to differentiate into numerous specialised cell types. The *in vivo* benefits of these have been demonstrated previously (86,87), although the exact culture conditions which would enable controlled differentiation are yet to be identified. However, their potential for tumorigenicity, the prospect of immune rejection and the ethical dilemmas associated with embryonic stem cells have limited their clinical application (85).

Adult stem cells are the most extensively investigated cell types in stem cell biology, regardless, progression has been slow. Despite this, study of adult stem cells is ongoing due to their extensive potential to be applied as therapies in a vast array of disorders. The most promising source of adult stem cells is adult bone marrow. The use of autologous adult stem cells avoids the obstacles associated with an immune response (88,89), which is useful for autologous and tissue-specific regenerative therapies. Within the past two decades, stem cells have been identified throughout the tissues of the body, not just the bone marrow (90). It has been indicated that stem cells may function as primary repair entities for the particular organ in which they reside. These tissue-specific progenitors are now the subject of numerous different areas of research (88).

Mesenchymal stem cells (MSCs) are derived from the bone marrow stroma. MSCs have been demonstrated to differentiate *in vitro* into a variety of tissue types, including smooth muscle, urothelium and endothelial cells. It is for this reason that bone marrow-derived MSCs are an attractive candidate for bladder tissue regeneration (91). Chung *et al* (92) reported positive results in healthy rat bladders following the introduction of porcine small intestinal submucosa seeded with MSCs from rat bone marrow. These rats exhibited normal urinary bladder architecture, and histological analysis demonstrated well-differentiated urothelial and smooth muscle cells compared with control experiments using unseeded intestinal submucosa (92).

Amniotic fluid-derived stem (AFS) cells, adipose-derived stem cells, and stem cells isolated from hair have all demonstrated the capability of differentiating into different urinary bladder cells *in vitro* (52,93,94). AFS cells were initially isolated for therapeutic use in 2007 and represent a minor subset of cells originating from the placenta and amniotic fluid (95). AFS cells have been demonstrated to expand extensively, doubling every 36 h (96). They have the advantage over embryonic stem cells in that they do not form tumours *in vivo* (95). AFS cells possess a differentiation potential between that of adult stem cells and embryonic stem cells and can be obtained from routine clinical amniocentesis, prenatal chorionic villus biopsies and placental biopsies performed after birth (95). Since their identification, these cells have been demonstrated to differentiate into functioning cell types of numerous different organs and to prevent bladder hypertrophy in cryo-injured mouse bladders *in vitro* via the regulation of post-injury bladder remodelling (97). AFS cells possess a wide range of potential applications in the field

of regenerative medicine, and may in theory supply the vast majority of the UK with suitable genetic matches for transplantation (98-100).

Stem cells are currently employed in tissue engineering using two main methods, including implantation of the stem cells *in vivo* without pre-differentiation and induction of stem cell differentiation towards the bladder *in vitro* followed by its implantation *in vivo* (101). The first method is often employed when a section of the bladder requires reconstruction, in comparison with the second method that is used when *de novo* whole bladder reconstruction is required.

Innervation is vital in bladder reconstruction. The autonomic nervous system controls the function of the bladder, thus a poorly functioning neuronal network can lead to significant bladder dysfunction (102). It has been indicated that seeding Schwann cells and neurotrophic factors may encourage nerve innervation of the urinary bladder (103).

Several bladder malformations exist, which are often identified prenatally with sonography. The future prenatal management of patients with bladder disease has emerged as a notable possibility (104). The potential to have readily available urological tissue at birth for a one-stage reconstruction following prenatal ultrasound-guided bladder biopsy is of note as this would present a significant advancement for the treatment of congenital urinary tract abnormalities as cells could be harvested and grown in transplantable tissue during gestation. This has been successfully demonstrated in foetal lambs (105). Chondrocytes obtained from hyaline and elastic cartilage were harvested from fetal lambs, expanded *in vitro* and seeded onto biodegradable scaffolds. The scaffolds were then implanted as replacement tracheal tissue in fetal lambs. Structural support and patency of the tissue engineered cartilage was maintained and all lambs that were allowed to reach term were able to breathe spontaneously. Thus, this may be useful for *in utero* repair of congenital tracheal abnormalities, such as tracheal atresia and agenesis (106,107).

## 5. Conclusion and perspectives

Existing data indicates a forthcoming role of tissue engineering disciplines in the management of urological diseases. With the extensive advancements within the field in previous years, the future of urology appears positive. However, the current knowledge of bladder reconstruction is insufficient in order to use it as a clinical standard in mainstream urological practice. Additional *in vitro* studies are required to make this possible. The most significant obstacle in material science is the equilibrium between the creation of biomaterials that are patient- and disease-specific and fit for purpose and a cost-effective manufacturing process. The primary objective in the field of cell biology and transplantation is not solely to control stem cell differentiation *in vivo* but also to be able to manage their growth following transplantation. The most significant aspect of cell type selection is the degree of regenerative potential; the more regenerative the cell is, the more likely it is to survive when implanted *in vivo*. The survival of artificial tissue following transplantation has been a significant obstacle thus far in current tissue engineering strategies. Other factors other than cell type selection are also important, however these factors often depend on the ability for the tissue to maintain initial growth and survive. Thus, as

the tissue grows a healthy self-sustained process of self repair is required, however, this will vary depending on the cell type used.

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