Minireview

Human urinary bladder regeneration through tissue engineering – An analysis of 131 clinical cases

Marta Pokrywczynska[1], Jan Adamowicz[1], Arun K Sharma[2,3,4,6] and Tomasz Drewa[1,5]

[1]Department of Tissue Engineering, Nicolaus Copernicus University in Torun, Ludwik Rydygier Medical College in Bydgoszcz, 85-092 Bydgoszcz, Poland; [2]Division of Pediatric Urology, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL 60611, USA; [3]Department of Urology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA; [4]Institute for BioNanotechnology in Medicine, Northwestern University, Chicago, IL 60611, USA; [5]Department of Urology, Nicolaus Copernicus Hospital, 87-100 Torun, Poland; [6]Department of Biomedical Engineering, Northwestern University, Evanston, IL 60208, USA
Corresponding author: Marta Pokrywczynska. Email: marta.pokrywczynska@interia.pl

Abstract

Replacement of urinary bladder tissue with functional equivalents remains one of the most challenging problems of reconstructive urology over the last several decades. The gold standard treatment for urinary diversion after radical cystectomy is the ileal conduit or neobladder; however, this technique is associated with numerous complications including electrolyte imbalances, mucus production, and the potential for malignant transformation. Tissue engineering techniques provide the impetus to construct functional bladder substitutes *de novo*. Within this review, we have thoroughly perused the literature utilizing PubMed in order to identify clinical studies involving bladder reconstruction utilizing tissue engineering methodologies. The idea of urinary bladder regeneration through tissue engineering dates back to the 1950s. Many natural and synthetic biomaterials such as plastic mold, gelatin sponge, Japanese paper, preserved dog bladder, lyophilized human dura, bovine pericardium, small intestinal submucosa, bladder acellular matrix, or composite of collagen and polyglycolic acid were used for urinary bladder regeneration with a wide range of outcomes. Recent progress in the tissue engineering field suggest that *in vitro* engineered bladder wall substitutes may have expanded clinical applicability in near future but preclinical investigations on large animal models with defective bladders are necessary to optimize the methods of bladder reconstruction by tissue engineering in humans.

Keywords: Clinical research, reconstruction, regeneration, urinary bladder, tissue engineering

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Introduction

Urinary diversion or augmentation cystoplasty are required for different clinical indications in adult and pediatric patients. The most frequent indication for urinary diversion is bladder cancer requiring cystectomy.[1] However, the most common underlying causes of bladder dysfunction that may require augmentation cystoplasty include small contracted bladder due to tuberculosis, radiation induced cystitis, or interstitial cystitis.[2] Detrusor hyperreflexia due to supra sacral spinal lesion, refractory detrusor instability, and poor bladder compliance are other bladder dysfunctions where augmentation cystoplasty may be indicated. Additionally, congenital or traumatic conditions may demand augmentation cystoplasty or urinary diversion.[3,4] The preferred modalities of treatment include (1) the preservation of the upper urinary tract; (2) urinary continence; and (3) adequate reservoir emptying with the simultaneous reduction of urinary tract infections.[5]

Urinary diversion can be categorized into two groups as either continent or incontinent. Non–continent ureteroileocutaneostomy is the most frequently used type of diversion. In this method, the ileal conduit is made from approximately 15 to 25 cm of pre–terminal ileum as urine is collected in an external pouching system. A second option utilizes the continent cutaneous pouch. The continent urinary reservoir is constructed from a detubularized bowel segment in which patients empty the reservoir by self-catheterization. The most common physiological method of the urine diversion following cystectomy is creation of the orthotopic neobladder. The neobladder is constructed from a portion of the patient's bowel and anastomosed to the patient's native urethra and ureters.[6,7] In order to construct the continent urinary reservoir or orthotopic neobladder, approximately 40–80 cm of pre-terminal ileum is used.[8] However, the use of a patient's own gastrointestinal tissue comes with severe complications, including bowel

anastomosis leakage at the site where part of the bowel was resected, sepsis, infections, mucus production, stone formation at the site of the neobladder, and metabolic abnormalities (acidosis). These complications are associated with severe morbidity and mortality.[9]

Augmentation cystoplasty is the surgical enlargement of the existing bladder using approximately 20 cm segment of the small bowel, large bowel, or less often stomach.[10]

Principles of tissue engineering

Tissue engineering is an interdisciplinary field that melds the principles of bioengineering and life sciences to create the biologic substitutes that restore, maintain, or improve tissue or organ function following insult.[11] There are two major approaches of organ or tissue reconstruction by tissue engineering. The first approach involves either natural or synthetic biomaterials that encourages the *in vivo* regenerative process by serving as a solid support matrix (scaffold) for the in-growth of native cells. The second approach involves biomaterials with a cell seeded approach that utilizes autologous sources of patient cells. The creation of this newly created ''neo-tissue'' is subsequently grafted back to the host for the completion of the regeneration process. The use of autologous cells reduces the fears of immune rejection as encountered when using allogeneic cell sources.

The ultimate goal of bladder tissue engineering is to identify scaffolding material as well as cell sources that would be able to recapitulate bladder wall components and provide greater physiological output than insulted native tissue. The ideal biomaterial for this purpose should be biocompatible, non-immunogenic, and biodegradable. Moreover, it should exhibit the appropriate mechanical parameters (elasticity, tensile strength) and maintain properties that allow it to function as a proper barrier against urine and infection.

Methods

Within this review, we have thoroughly perused the literature using PubMed in order to identify clinical studies involving bladder reconstruction utilizing tissue engineering methodologies.

Results

Lessons from the past

The initial clinical experiments that can be considered as prototypes of research utilizing tissue engineering strategies for the regenerating bladder were conducted in the 1950s. The materials used for bladder reconstruction were intended to initiate spontaneous regeneration.[12,13]

In 1957, Bohne *et al.* used plastic molds for bladder reconstruction in seven patients following subtotal cystectomy. Indications for the cystectomy included severe, chronic interstitial cystitis ($n=2$), diffuse bladder carcinoma ($n=4$), or small contracted bladder in a patient as a consequence of exposure to tuberculosis ($n=1$). The plastic mold was constructed in the shape of the bladder and was implanted orthotopically for several weeks and then subsequently removed. The generated ''pseudo-bladder'' consisted mainly of fibrotic tissue and underwent contraction over time. Other complications associated with the use of plastic molds were vesicoureteral reflux, dilatation of the upper urinary tract, recurrent urinary tract infections, and eventual deterioration of renal function. This experiment failed in all cases.[12] Other clinical experiments using plastic implants as a temporary bladder substitute after cystectomy were reported in 1958 and 1964.[13,14] Since these trials were also associated with a number of postoperative complications and high mortality, this technique was eventually abandoned. A noteworthy observation arising from these early research studies is that the urothelium has the propensity to regenerate completely, even migrating from the ureters following radical cystectomy. This is the result of the high proliferative potential of epithelial cells and their capacity for self-renewal. There was no evidence of bladder smooth muscle regeneration in any of the 36 patients treated with this method (Table 1).

An alternative material used for bladder reconstruction was a gelatin sponge treated with an alcohol or a synthetic resin (nobecutane). Nobecutane sprayed on a wound formed a plastic film which served as a dressing material. It contains specially modified acrylic resin in an organic solvent (ethyl acetate) and TMTD (tetramethylthiuram disulphide) which is strongly bactericidal and fungicidal.[15] Nobecutane spray was used as a temporary dressing of skin grafts or surgical wounds.[15–17] Gelatin sponge treated with an alcohol or nobecutane was used for both augmentation cystoplasty and bladder replacement following subtotal cystectomy. Gelatin sponge provided a temporary scaffold for tissue growth, underwent remodeling and degradation over time. Tsuji *et al.* used gelatin sponges for bladder reconstruction in four patients after total or subtotal cystectomy ($n=2$ in each case) due to bladder cancer. Although new bladders with a capacity of 80–100 cc were obtained as early as 1–2 months, every patient in this study had moderate to severe urinary incontinence. Other complications related to this method were urine leakage, vesicoureteral reflux, and defective ureteral orifices. Furthermore, the new bladders decreased capacity progressively and eventually succumbed to contractability issues. Consequently, urinary re-diversion became necessary in all cases.[18]

Better results were obtained if gelatin sponges that were sprayed with nobecutane or rezifilm (a plastic dressing spray for surgical wounds) were utilized for bladder augmentation. This experiment was carried out on a group of five patients: four tuberculosis contracted bladders and one congenital hour-glass bladder. The procedure was successful in four cases and failed in one case. One patient increased bladder capacity from 50 cc to 250 cc after 1 month and 350 cc after 8 months. Cystography showed a normal-shaped bladder and no reflux. Biopsy of the new bladder wall showed a complete epithelial covering and excellent muscle regeneration without inflammatory reaction. The patient was completely relieved of urinary complications and voided without residual urine. According to the authors, failure in one patient was associated with significant fibrosis, and thereby losing regenerative capacity.[19] Despite the more than satisfactory results, the fact that the gelatin sponge was not used in further clinical trials

Table 1 Biomaterials and artificial tissues used for human bladder tissue engineering (131 cases)

Biomaterial/ artificial tissue	Indication for bladder reconstruction	No. clinical cases	Regeneration degree (time) (no. cases)		Shrinkage	Complications	Outcome
			Urothelium	Muscle			
Plastic mold[12]	Interstitial cystitis Bladder cancer Small contracted bladder (tuberculosis)	7	Autolysis (53 days) ($n=1$)	No (53 days) ($n=1$)	Regenerated bladder contracted down over time	Pyelonephritis, septicemia vesicoureteral reflux, dilatation of the upper urinary system, calculus-pyohydronephrosis	Failed
Plastic mold[13]	Bladder cancer	1	Complete (6 weeks)	No (6 weeks)	No data	No data	Questionable
Plastic mold[14]	Bladder cancer	28	Complete (no data)	No (no data)	No data	Vesicoureteral reflux, widening of the renal pelvis	Questionable
Gelatin sponge[18]	Bladder cancer	4	NA	NA	Bladder decrease in capacity progressively to became a contracted bladder	Urinary incontinence, new defective ureteral orifices, vesicoureteral reflux	Failed ($n=4$)
Gelatin sponge +nobecutane[19]	Small contracted bladder (tuberculosis) congenital hour-glass bladder	5	Complete (8 months) ($n=1$)	Satisfactory (8 months) ($n=1$)	No data	Hydroureteronephrosis urinary leakage	Good ($n=4$) Failed ($n=1$)
Japanese paper + nobecutane[20]	Small contracted bladder (tuberculosis, interstitial cystitis)	13	Complete (3–5 months)	Almost complete (9 months)	No data	No data	Good ($n=11$) Failed ($n=2$)
Japanese paper + nobecutane[21]	Small contracted bladder (tuberculosis) Bladder cancer	4	NA	NA	Reconstructed bladder decrease in capacity	Urinary leakage Vesicoureteral reflux	Good ($n=3$) Failed ($n=1$)
Formalin preserved dog bladder[22]	Small contracted bladder (tuberculosis)	2	NA	NA	No data	Vesicoureteral reflux	Good ($n=1$) Poor ($n=1$)
Formalin preserved dog bladder[18]	Bladder cancer	10	NA	NA	New bladder decrease in capacity progressively to became a contracted bladder,	Urinary incontinence, defective ureteral orifices, vesicoureteral reflux	Good ($n=1$) Failed ($n=9$)
Lyophilized human dura[24]	Small contracted bladder (interstitial cystitis, irradiation cystitis) Bladder cancer	34	Complete	No sign of muscle regeneration (2–6 years)	No data	Perforation	Good ($n=19$) Failed ($n=10$) Unknown ($n=5$)
Lyophilized human dura[25]	Neurogenic bladder (spinal trauma, myelomeningocele)	10	Complete	Week (1 year)	No data	No complications	Good ($n=10$)
Bovine pericardium[26]	Enterovesical fistula	1	NA	NA	No data	No complications	Questionable ($n=1$)

(continued)

Table 1 Continued

Biomaterial/ artificial tissue	Indication for bladder reconstruction	No. clinical cases	Regeneration degree (time) (no. cases)		Shrinkage	Complications	Outcome
			Urothelium	Muscle			
Small intestinal submucosa[32]	Bladder extrophy	5	Complete (6–18 months)	Partial (6–18 months)	No data	No complications	Poor ($n=5$)
Bladder acellular matrix seeded with urothelial and smooth muscle cells[31] (Atala, 2006)	Neurogenic bladder (myelomeningocele)	4	Complete (31 months)	Complete (31 months)	Absence	No complications	Questionable ($n=4$)
Composite: collagen + polyglycolic acid seeded with urothelial and smooth muscle cells[31]	Neurogenic bladder (myelomeningocele)	3	Complete (31 months)	Complete (31 months)	Absence	No complications	Good ($n=3$)

indicates that some doubts persisted regarding the reliability of the presented results. ''Great'' muscle regeneration that was observed by the authors appeared unlikely due to the poor regenerative capacity of muscle tissue. Cellularization of the $9 \times 9 \times 10$ cm graft by muscle cells migrating from the surrounding native bladder tissue was unlikely, especially in the case of contracted bladder, in which the number of muscle fibers is significantly reduced. Moreover, later studies showed that during the healing process, the spongy material disintegrated into small pieces and fell off. However, small portions of the material adhered to the granulated tissue and incorporated into the reconstructing bladder wall. The nobecutane-treated gelatin was no longer gelatin but acted like synthetic resin and did not degrade even after two years. This lead to calculi formation and other undesirable side-effects.[20]

Japanese paper sprayed with nobecutane was also used for bladder augmentation in 13 patients with small, contracted bladders due to various pathologic conditions. Dome-shaped implants of ~8–10 cm in largest diameter and 3 cm in height were made by using Japanese paper produced from the rice paper plant (*Tetrapanax papyrifer)* sterilized and sprayed with nobecutane. This material provided a temporary scaffold for tissue growth and was completely removed transurethrally after 1 month. Favorable results were obtained in 11 patients with tuberculosis-stricken contracted bladders. These patients regained normal bladder capacity and voiding functions through the urethra. The bladder capacity increased from ~3–70 ml prior to implantation to ~200–300 ml in 1–5 years following reconstruction. Five months postoperatively, the bladder appeared to be approaching normal morphology. There was no improvement in two patients suffering from interstitial cystitis.[20] Another clinical experiment using Japanese paper for bladder reconstruction was performed in 1978 by Fujita *et al.*[21] Urinary bladder reconstruction was performed in four patients with either tuberculosis ($n=2$) or bladder cancer ($n=2$). Japanese paper (20 cm^2 in diameter) sprayed with nobecutane was used for bladder reconstruction. Satisfactory results were obtained in three patients. One patient with long standing history of contracted bladder failed to acquire an adequate capacity.[21] However, it should be noted that the short-term three-month follow-up of this study does not allow for evaluation of treatment effects and to draw clinically relevant conclusions. Despite these preliminary, encouraging results, no further clinical studies have been performed.

Tsuji *et al.* used formalin preserved dog bladder for bladder reconstruction in 10 patients with bladder cancer after sub-total or radical cystectomy.[22] The formalin preserved bladder was used as a temporary scaffold for tissue growth and was removed 2–3 weeks after the procedure. One patient whose lower half of bladder including posterior urethra was reconstructed with preserved bladder maintained a good general condition with satisfactory bladder function, except for slight stress incontinence. The final results of the bladder reconstruction in other patients after subtotal cystectomy were not

so good and consequently urinary diversion was carried out 6–8 months later. The results of bladder reconstruction following total cystectomy were disappointing as well. Although a new ''pseudo-bladder'' was obtained in relatively short time, it decreased in capacity thereafter. Furthermore, stricture of the new ureteral orifices, urinary incontinence, and vesicoureteral reflux occurred frequently.[22]

Another biomaterial used for bladder reconstruction was lyophilized human dura.[23] In 1975, Kelami *et al.* described cystoplasty using dura mater in 34 patients with good results. There were two main indications for bladder reconstruction-contracted bladder ($n = 6$) and bladder wall resection due to bladder carcinoma ($n = 28$). Lyophilized human dura 6×14 cm in size served mainly as a matrix for tissue growth. Dura was completely absorbed after 10–12 weeks and follow-up ranges were from 2 to 6 years. Preoperative contracted bladder capacities ranged between 60 and 80 ml. Postoperatively, all six patients had a capacity 2–3 times larger than before. One patient had a preoperative capacity of 30 ml which increased to 180–220 ml postoperatively while nine patients (26.5%) had a cancer recurrence during the first postoperative year. Lastly, one patient died on the 14th postoperative day. During postoperative cystoscopy, the dura was perforated, resulting in peritonitis. Five patients were lost to follow-up during the third postoperative year but they had no previous signs of recurrence. Thirteen patients (46.4%) had no recurrence 2–6 years after surgery. They maintained large bladder capacities and emptied them without residual urine. Cystoscopically, there were no differences in the appearance between regenerated and native epithelium. The reconstructed bladders were well vascularized and there were no signs of contraction. However, there were no signs of smooth muscle regeneration at autopsy of patient who died during follow-up.[24]

In 1995, Arikan *et al.* described augmentation cystoplasty using dura in neurogenic bladder dysfunction. The etiology of bladder dysfunction was spinal trauma ($n = 7$) or myelomeningocele ($n = 3$). Human skull dehydrated dura mater 6×14 cm in size was used for the cystoplasty. Ten patients suffering from neurogenic bladder dysfunction underwent a modified Bramble-Clam augmentation. The follow-up period was 28 months in which 7 of 10 patients were completely continent. The urodynamic parameters such as cystometric capacity and intravesical pressure were improved. The histological examination of the punch biopsies revealed normal transitional epithelium. However, again only weak smooth muscle regeneration was evident in the form of irregularly shaped muscle bundles.[25]

Moon *et al.* reported a case of enterovesical fistula repair using bovine pericardium. The patient involved in this study had a history of radiation, several laparotomies, and maintenance of a chronic indwelling urethral catheter. The defect after resection of the fistula site was large; therefore, it was impossible to close the bladder without augmentation. Cystoplasty using the intestine was contraindicated due to the poor condition of the intestine because of previous radiation therapy. The bladder wall defect was repaired using Supple Peri-Guard® (Synovis) prepared from bovine pericardium.[26] The results were questioned since the reconstructed area was very small ($2.4\,\text{cm} \times 2\,\text{cm}$) and the authors provided no data for bladder capacity and compliance. Further studies are needed to identify the safety and effectiveness of bovine pericardium as a graft material for bladder augmentation.[26]

These failures of human bladder regeneration studies stems from the limited capacity for regeneration of adult mammalian tissues. Reconstruction of the urinary bladder requires smooth muscle regeneration because its function as a urine reservoir depends mainly on compliance and contractility of detrusor muscle. Independent observations of adult healing following injury have shown that in the majority of organs, explanted epithelial tissues and basement membranes regenerate spontaneously following excision while some elements of stroma does not.[27] The aforementioned clinical trials confirmed that urothelium regenerates spontaneously, while the smooth muscle compartment heals via repair through scar formation. Numerous experimental studies indicated that smooth muscle regeneration in adult mammals can be induced, but requires tissue-engineering techniques.[28–30]

New concepts for bladder regeneration

The rapidly emerging field of tissue engineering holds great promise for the generation of functional tissue substitutes which could be used in reconstructive urology. The first clinical trial for tissue engineering in urology using cell seeded grafts was published in 2006. It involved the engineering of human bladder wall for young patients with myelomeningocele and end-stage bladder disease.[31] Seven patients with a mean age 11 years, with high pressure, poor bladder compliance due to myelomeningocele participated in this trial. The bladder wall substitute was created *in vitro* from collagen or a composite of collagen and polyglycolic acid (PGA) and autologous urothelial and smooth muscle cells. Bladder biopsy sample ($1–2\,\text{cm}^2$) was obtained from the bladder dome of each patient. Urothelial and smooth muscle cells were isolated and cultured through 7–8 weeks. Cells were seeded at density of 50×10^6 per cm^3 on the bladder mold and ranged from $70\,\text{cm}^2$ to $150\,\text{cm}^2$ and was created from collagen and PGA ($n = 3$) or decellularized bladder submucosa ($n = 4$). The tissue engineered bladder construct was implanted either with or without an omental wrap. The mean follow-up was 46 months (22–61 months). This study indicated that the use of composite scaffold made of collagen and PGA led to better bladder wall regeneration compared to collagen scaffold alone. Also, omental wrapping showed to be beneficial. Omentum enhanced vascularization of grafts due to its rich blood supply. The two patients in which bladders were reconstructed with PGA-collagen cell seeded scaffolds with omental coverage showed increased compliance, decreased end-filling pressure, increased capacities, and longer dry periods over time. The bladder biopsy revealed proper morphology and architecture of the reconstructed bladder wall. The line between tissue engineered and native bladder wall was grossly, indistinguishable.[31] It should be emphasized that satisfactory results were obtained only in two patients (28.6%). The majority of patients did not achieve

good bladder capacity and compliance. It means that reconstructed bladder wall consisted of fibrous tissue. Due to this, patients were forced to perform clean intermittent catheterization. This small pilot study indicated that engineered tissues can be implanted safely; however, further experimental and clinical studies are required especially to achieve functional bladder.

Recently, collagen acellular matrix was used for bladder augmentation in five extrophic patients with mean age 10.4 years.[32] The patients enrolled in this study presented poor bladder capacity and compliance after complete extrophy repair. Small intestinal submucosa membrane (SIS, Surgisis®, Cook Urological Spencer), 5×4 cm in size was fashioned into diamond shape and grafted to the bladder. The suture line was sprayed with fibrin glue and augmented bladder was covered by soft perivesical tissue or by an omentum flap. Bladder capacity and compliance following cystoplasty increased slightly at 6 months and remained stable 18 months after surgery. Bladder biopsy performed six months after augmentation indicated normal transitional mucosa and sero-muscular layer containing sparse smooth muscle fibers, small nerve trunks, and vessels. SIS was absent at this period in time. Summarizing, the bladder regeneration was feasible in these patients, but bladder capacity and compliance was poorly increased to obtain significant clinical benefit.[32] It is interesting that such trial was conducted despite that the experiments on animal models demonstrated no increase in capacity following cystoplasty with acellular collagen.[30,33,34].

Future perspectives

Advances in the tissue engineering hold great promise for reconstructive urology. Over the last two decades, several bladder wall substitutes have been introduced. Numerous experimental studies confirm that presence of cells is necessary to obtain adequate tissue structure and function of the reconstructed bladder. Cells implanted together with the scaffold play various functions: strengthen mechanical properties of the scaffold; acting as an impermeable barrier to urine; and stimulate scaffold remodeling while secreting trophic factors which enhance the process of regeneration.

Autologous bladder cells can be harvested, expanded *ex vivo*, and seeded into the scaffold and transplanted back into the host. It was demonstrated that urothelial and smooth muscle cells from patients with the neurogenic bladder can be effectively expanded *ex vivo* and used for bladder augmentation,[31] but in the light of previously published experimental works it seems questionable. However, it was also indicated that smooth muscle cells isolated from neuropathic bladders have shown abnormal growth, less contractile ability, and inferior adherence compared to normal controls.[35] Moreover, the profile of gene expression in neuropathic bladder smooth muscle cells is altered.[36] Hence, smooth muscle cells derived from diseased bladders may not be appropriate for tissue engineering purposes. Autologous urothelial cells from patients with interstitial cystitis or other form of chronic cystitis, neuropathic bladder, posterior urethral valves, epispadias, and non-neurogenic bladder dysfunction have reduced capacities for proliferation and differentiation *in vitro*.[37–39] Muscle invasive bladder cancer is a separate category of disease in which autologous bladder cells cannot be used for bladder reconstruction. It must be emphasized that this is the largest group of urological patients requiring the lower urinary tract reconstruction.[40] Also for elderly patients, a bladder biopsy may not yield enough normal cells for expansion and transplantation. These findings clearly indicate a need to identify alternative cell sources for bladder tissue engineering.

Stem cells offer great promise when autologous bladder cells cannot be used. Potential sources of autologous stem cells for bladder tissue engineering are bone marrow, adipose tissue, skin, and hair follicles.[41,42,28,43] Mesenchymal stem cells are capable of differentiating into multiple cell types, among them smooth muscle cells, urothelium, endothelial cells, and neurons.[44–46]

There are two methods commonly employed in bladder tissue engineering: implantation of stem cells *in vivo* without pre-differentiation or induction of stem cell differentiation toward the specific target cells *in vitro* followed by implantation *in vivo*.[44] It is believed that the host tissue environment directs the fate of the stem cells to bladder cells. This method is usually used when a half of the bladder is reconstructed or bladder wall is augmented with the stem cell seeded graft. Mesenchymal stem cells influenced by trophic factors secreted by smooth muscles or urothelium from surrounding bladder tissue differentiate into the smooth muscles cells or urothelial cells. However, when the tissues are affected with disease, the implanted non-induced stem cells may fail to differentiate to the wanted target cells. The second approach utilizes the concept of whole bladder construction *de novo* or reconstruction of large defects requiring the differentiation of stem cells into smooth muscle phenotype *in vitro*. The question remains whether the induced cells can fully differentiate into the specific cell type *in vitro*.

Numerous studies have shown that using unseeded grafts for urinary bladder reconstruction leads to fibrosis and shrinkage, while addition of cells prevents this complication. In our previous study, we have found that MSCs change the profile of cytokine expression in reconstructed urinary bladders. MSCs activated within the environment of the injured urinary bladder have been shown to up-regulate anti-inflammatory cytokines, prevent fibrosis, and enhance smooth muscle regeneration.[42]

Although partial or complete regeneration of smooth muscles is usually observed in cell-seeded grafts, their morphology and function are not completely equivalent to native bladder smooth muscles.[47] Perhaps the application of electrical stimulation in cell culture *in vitro* will induce proper smooth muscle fibers arrangement and function *in vivo*. However, even if regenerated SMCs are physiologically compromised as compared to their native SMC counterparts, they may be still sufficient to provide urinary bladder wall compliance. This is in contrast to ileal neobladders which do not generate coordinated contraction but provide adequate low-pressure storage capacity.

One of the most challenging issues of functional bladder regeneration is innervation.[48] The storage function of the urinary bladder depends entirely on the autonomic nervous system. Thus, the poorly regenerated neuronal network within the artificial urinary bladder wall can lead to urinary bladder dysfunction after such a surgery. It was indicated that Schwann cell seeding or the application of exogenous neurotrophic factors may induce bladder innervation.[49,50] The ultimate goal of bladder tissue engineering is the construction of physiologically functional bladder tissue *de novo*. However, it should be noted that even the construction of a passive reservoir for urine utilizing tissue engineering techniques instead ileal segments carries significant benefits. In this setting, the innervation of reconstructed bladder is not necessary, as long as the reconstructed bladder retains the shape and position that could allow for adequate emptying.

One of the major barriers for survival of a large graft is vascularization. It was reported that omental coverage, endothelial cell seeding or application of exogenous angiogenic factors enhance capillaries ingrowth to the graft, but still may be insufficient to provide robust vascular supply to sustain a large graft.[30,51–54]

Another major problem is that all preclinical animal studies concerning bladder regeneration are typically performed in healthy bladders. In such a research model, compensative expansion of the native bladder can occur even when the growth of regenerated bladder tissue is insufficient.[55] Consequently, if total bladder function is set as the final outcome, it cannot be concluded if it is a result of compensative expansion or bladder augmentation.[56] Furthermore, in numerous experimental studies the reconstructed defect is too small, follow-up is too short or experimental group too small to draw meaningful conclusions.[57,58]

Recent progress in the tissue engineering field suggest that *in vitro* engineered bladder wall substitutes may have expanded clinical applicability in near future but preclinical investigations on large animal models with defective bladders are necessary to optimize the methods of bladder reconstruction by tissue engineering in humans.

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