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Regenerative Medicine in Bladder Reconstructive Surgery

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Abstract

This article explores the options for bladder reconstructive surgery and the role of regenerative medicine and tissue engineering. The indications for bladder reconstructive surgery are explored to complement medical management, which remains the first line for bladder dysfunction. Different strategies for research in tissue engineering of the bladder are discussed.

Patient summary: Options for bladder reconstructive surgery are discussed, including regenerative medicine and tissue engineering.

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1. Introduction

The bladder is a complex organ with specialised functions of storage and volitional voiding of urine mediated by spinal reflex mechanisms involving sympathetic and parasympathetic neural pathways, respectively [1–3].

Storage of urine delivered by the kidneys is combined with maintaining its electrolyte composition via passive permeability and active ion transport [4]. The urothelium separates the bladder muscle from the urine and has mechanosensory attributes, and thus responds via endocytosis and exocytosis to physical and chemical changes such as intravesical pressure and urinary electrolytes [5,6].

2. Bladder dysfunction and its consequences

The pattern of normal reflex voiding can be disturbed by bladder and/or sphincter dysfunction. Any structural, neurogenic, or functional abnormality of the bladder, structural or functional outflow obstruction, or abnormal uroflow dynamics, as in severe vesicoureteric reflux, can have significant consequences along the entire urinary tract

[7–9]. Some of these anomalies, such as posterior urethral valves [10,11], myelodysplasia, sacral agenesis, spinal tumours, and exstrophy of the bladder, can have a chronic impact causing detrusor hypertrophy and fibrosis, change the bladder from a highly compliant, supple muscular organ to a stiff and noncontracting organ with poor compliance, endangering the upper urinary tract and resulting in renal impairment or failure. In such circumstances, management options might involve replacing diseased portions of the bladder [12,13].

The natural history of the urinary tract in neurogenic bladder sphincter dysfunction that is not satisfactorily managed is one of deterioration in the vast majority of cases within 3 yr [14].

Management of bladder dysfunction is aimed at protecting the upper tract while imparting continence, with medical options the mainstay towards these goals, including anticholinergics [15], clean intermittent catheterisation (CIC) [16–18], and Botox injections into the detrusor [19,20]. Urodynamics plays an important part in decision-making [21,22]. In individuals for whom catheterisation per urethra is not possible, surgical creation of a

Table 1 – Pros and cons of each bowel segment in bladder augmentation

Bowel segment	Pros	Cons	Refs
Urothelium-based	No cancer risk No metabolic changes	Unpredictable outcome	[31–34]
Colon	Easy to harvest Minimal metabolic changes	Cancer risk	[39,40,42]
Ileum	Easy to harvest Long experience Low cancer risk	Mucus Metabolic factors Perforation?	[30,40,43,44]
Stomach	Metabolic factors	Acidity Cancer risk	[36,37,45]

catheterisable channel via the Mitrofanoff concept facilitates CIC [23–26].

Portions of the diseased bladder may have to be replaced when medical management is insufficient, as in end-stage bladder disease.

3. Bladder augmentation

Urothelial-based strategies for augmentation include ureterocystoplasty (UC) and detrusorotomy with or without a seromuscular colonic patch (autoaugmentation, AA). UC is only appropriate in selected clinical scenarios and is not an option in most circumstances [27–30]. AA needs to be performed early to be effective and concerns regarding failure with limited success have been reported in isolated series, so AA is not an universally accepted option [31–34].

Enterocystoplasty is the most widely accepted procedure for bladder augmentation, with the ileum being the favoured segment and the most compliant [35]. Gastrocystoplasty is a less popular option because of unfavourable side effects [36,37]. However, incorporation of bowel segments into the urinary tract has some undesired consequences, primarily because the gut is structurally and physiologically not suited for prolonged exposure to urine [38].

Gut produces mucous [39] and with it consequences [40] of bacteriuria, recurrent infections, stones, metabolic changes in the long term, and unknown cellular effects including malignancy [41]. Table 1 highlights the pros and cons for each type of bowel segment in bladder augmentation.

4. Regenerative medicine and the bladder

Regenerative medicine involves diverse areas of tissue engineering, stem cells, and cloning with the common goals of “replacing or regenerating human cells, tissues or organs, to restore or establish normal function” [42]. It offers the possibility to replace old and damaged cells with genetically compatible young and functional cells [43].

Tissue engineering is multidisciplinary and combines the principles of cell transplantation, materials science, and engineering to construct functional tissues and supplement or replace diseased and defective body parts [44,45].

Ideally, a neoengineered bladder must be able to mimic filling and voiding as in normal bladder function. However,

this is extremely complex and appears to be very distant in prospect. Therefore, a compliant neobladder with a good functional barrier (realising low pressure storage) but drained via CIC (replacing voiding function) could be a realistic prospect in the coming years.

5. Urothelial tissue engineering

Native urothelium is a quiescent tissue for which the turnover of cells is extremely slow, with few cells if at all in cycle. However, in response to injury, the urothelium adopts a proliferative wound-healing phenotype with high regenerative capacity in attempting to re-establish an effective urinary barrier [46]. This characteristic of the urothelium has been exploited in regenerative medicine laboratories, where urothelium can be freely grown via passaging.

For tissue culture to be considered successful, the cultured urothelium must bear a similar footprint to its native equivalent, meaning it should look and act the same as native epithelium. Lewis [4] outlined these features, including high transepithelial resistance (TER) (native tissues have TER >20 000 ohms/cm²); low permeability to water and urea; composition of three distinct cells with an asymmetric unit membrane (AUM); expression of markers of terminal differentiation such as UPK III at the apical surface; and the presence of tight junctions.

Proliferative normal porcine urothelium is considered to be “leaky” in that it is a monolayer with poorly differentiated phenotype, thus allowing passage of urine to the suburothelial zone. Cross et al [47] showed that manipulation of culture conditions, such as inclusion of bovine serum, can induce normal human urothelial cells to propagate in vitro and form a urothelium with barrier properties [47].

6. Bladder tissue engineering strategies

Most strategies involve the use of a scaffold or matrix to support the development of new tissues. Scaffolds can be natural or synthetic. Natural materials can be in their original form, such as amniotic membrane [48]; in a processed form, such as bladder acellular matrix [49,50]; or in elemental form, such as collagen [51]. Synthetic scaffolds are typically made of poly-glycolic acid (PGA) or poly-lactic glycolic acid (PLGA) as materials approved by the US Food and Drug Administration [45].

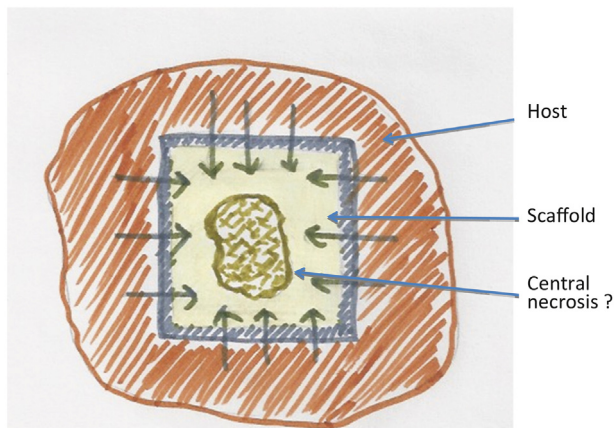


Fig. 1 – Schematic illustration of a scaffold relying entirely on host tissue. Cell infiltration occurs from outside to inside, as depicted by the green arrows. Infiltration into the central area depends on the size of the patch, the surface area, and its ratio to the perimeter; the health of the native tissue; and the type of cells that infiltrate into the patch.

Natural scaffolds may retain natural growth factors that could play a vital role in tissue integration, but their natural architecture can be limited by the source of the tissue. By contrast, synthetic scaffolds can be shaped to meet requirements via modification of their physical properties, and thus have potential as a readily available supply suitable for commercial purpose and value.

A scaffold or matrix can be used simply as an implant on its own, depending then entirely on the host to provide the ingredients to develop new tissue. Some researchers have seeded scaffolds with cultured cells from the organ of interest to promote regeneration.

The limitation of scaffolds alone that rely on host tissue to regenerate (Fig. 1) involves the size of the patch and the ratio of the perimeter to the surface area [44]. Other factors influencing integration of a patch is the health of the native host tissue and infiltration by the right type of cells to form functional tissue. If the tissue is nonregenerative and the only cells infiltrating are fibroblasts, then it is unlikely that a functional tissue will form. Akbal et al [52] demonstrated that animals with bladder outlet obstruction (BOO) showed fibrosis and incomplete smooth muscle generation in their BOO model compared to normal bladders.

As a rule, seeded constructs perform much better than unseeded constructs, as demonstrated by a study in a canine model of subtotal cystectomy, in which a synthetic polymer seeded with autologous urothelial and smooth muscle cells (SMCs) resulted in tissue formation similar to the native bladder [53].

7. Bioreactor

Urothelial cells in culture are immature, although altering the environment can influence their terminal differentiation. The physical environment is just as important as the chemical conditioning [45]. To improve the biomimetic properties of bladder tissue generated in vitro, the physical environment of the bladder must be simulated [54]. In

functional tissue engineering, scaffolds seeded with cells are conditioned in an external bioreactor. The bioreactor manages the physical environment by controlling the nutrition and providing appropriate mechanical stimulation.

Bioreactors can be *in vivo* or *in vitro* types. An example of *in vivo* bioreactor is the omentum, which is very popular with researchers because of its enriched blood supply [55,56]. The omentum wraps around the construct with minimal inflammation, as confirmed by histology. However, the limiting factor could be its availability in an individual patient.

Use of *in vitro* bioreactor is another strategy for propagation of phenotypic mature tissue before its use *in vivo*. Farhat and Yeger [57] developed an *in vitro* bioreactor, which creates cyclical pressure increases with a view to mimic condition in the bladder. The bladder fills with urine passively and recycles several times a day, with rapid changes in volume. This is managed at the level of the superficial cells of the urothelium by mobilising the AUM containing cytoplasmic vesicles in response to stretch and maintaining the surface area as required [6].

8. Reseeding scaffolds

Oberpenning et al [58] used tissue engineering to create a functional canine *de novo* bladder. Autologous urothelial cells and SMCs were passaged *in vitro* and then seeded on either side of a polymer in the shape of a bladder and transplanted over an area above the trigone. The authors claimed that the capacity, compliance, and histology of their neobladder were similar to those of native tissue.

This reseeded approach with engineered urothelial cells and SMC was translated into humans, culminating in a report by Atala et al [59] with much promise and media hype. In this study, nine patients with neuropathic bladder were recruited. Bladder biopsies were taken and autologous SMCs and urothelial cells propagated *in vitro*. They were seeded onto collagen or collagen-coated PGA scaffolds in an incubator, first with SMCs and then with urothelial cells 48 h later. Cystoplasty was then performed on the patients with the neoengineered bladder; the interval between biopsy and reconstructive surgery was 7–8 wk. This was indeed an impressive scientific and surgical achievement, but early results were only reported for seven patients, with two patients apparently lost to follow-up. This raised much speculation as to how could patients have been lost in such a high-profile study.

Further in-depth analysis showed that although histology of full-thickness biopsies demonstrated all the components of the urothelium and detrusor, the postoperative bladder capacity and compliance achieved were modest. In addition, one of the seven patients with follow-up had to undergo revision enterocystoplasty. It took a long time for further follow-up data to emerge; in 2014, Joseph et al [60] presented results from a Tension-sponsored phase 2 clinical trial. The results were much less positive and attracted less attention. An increase in compliance was observed in four patients at 12 mo and in five patients at 36 mo, although the

difference was not clinically or statistically significant. There was no clinical or statistical improvement in bladder capacity at 12 or 36 mo in any patient. Adverse events occurred in all ten patients, although most were easily treated. Two patients had low cell growth following bladder biopsy, of whom one withdrew from the study and one underwent a second biopsy. Serious adverse events involving bowel obstruction and/or bladder rupture occurred in four patients. The authors concluded: “Our autologous cell seeded biodegradable scaffold did not improve bladder compliance or capacity, and our serious adverse events surpassed an acceptable safety standard” [60].

9. Composite cystoplasty

Composite cystoplasty is a different strategy that our group has been working on for a considerable number of years. The idea is to propagate autologous urothelial cells in vitro and then transfer them onto a vascular smooth muscle substrate that has been de-epithelialised. The main difference is that only the urothelium needs to be engineered; the smooth muscle tissue is borrowed from an existing preformed vascularised tissue in vivo. In clinical practice, the smooth muscle component of an enterocystoplasty rarely gives rise to any problems, unlike the bowel mucosa. By engineering the urothelium, the detrimental effects of the intestinal epithelium and its consequences described earlier can be avoided.

It is important to mention the importance of significant advances in cell culture techniques that allow the generation of consistently large numbers of cells sufficient for therapeutic use [61,62].

The first study of composite cystoplasty was performed in a porcine model in which five animals were used for proof of principle [63]. Autologous cells were harvested and grown in culture in vitro and then transferred to de-epithelialised uterus; the animals were then sacrificed at 3 mo after reconstruction. All animals voided spontaneously and the bladder looked macroscopically normal, with an increase in patch size, confirming success in terms of creation of a functional augmentation. However, detailed analysis at the cellular level showed areas of incomplete coverage, stromal inflammation, and reactive squamous changes. These changes were seen in the augmented segment and native bladder. The hypothesis was that proliferative rather than differentiated urothelium was used in this experimental study, and the poor urinary barrier of the proliferative tissue could have contributed to this problem.

In subsequent studies we combined in vitro propagated autologous urothelium with a vascularised de-epithelialised colonic pedicle produced via extraluminal dissection [64]. Extraluminal dissection was originally described by Hafez et al [65], who hydrodistended the bowel and performed the dissection from outside. We adapted this technique and inserted a Foley catheter into the lumen.

We demonstrated in our study that extraluminal dissection of the mucosa from its seromuscular base with the aid of an inflated Foley catheter facilitates the creation

of robust seromuscular segments with no colonic epithelial regrowth in a porcine model. The Foley catheter behaves like a mucophilic device, attracting the mucosa from within, thus allowing access to the correct plane of dissection. The results indicate that a differentiated urothelium combined with host colon-derived smooth muscle successfully forms a functional augmented bladder and effectively overcomes the problems of mucus and calculi seen in conventional EC, without introducing a propensity for fibrosis or contraction [66].

10. Tissue engineering potential for diseased bladders

In common with all experimental models, tissue engineering methodology is first attempted in animals with normal bladders to establish proof of the principle. Therefore, before any of this work is translated into humans, we need to evaluate the growth potential of urothelium harvested from diseased human bladders and the differentiation capacity of the cells.

Despite considerable progress in the development of robust techniques to culture and differentiate urothelium from surgical samples of normal bladder, our most recent work demonstrated that urothelium from abnormal (neuropathic and non-neuropathic) bladders has a compromised capacity for proliferation and differentiation in vitro [67]. The inference from this study is that we either have to “rejuvenate” these cells or find an alternative cell source.

The basis for this compromised phenotype is unknown, but poses a risk to translating composite enterocystoplasty into clinical practice. One possible hypothesis is that the modification to the urothelium is epigenetic, caused by the diseased, chronically inflamed environment of the bladder. Such heritable changes are supported by the fact that despite being removed from the adverse, diseased bladder of the donor, a compromised phenotype persisted through subsequent expansion and differentiation in vitro. If such epigenetic changes have occurred, it is possible that epigenetic modifying agents might reverse compromise to the urothelium [68] via related mechanisms. An ability to take compromised urothelium from diseased bladders and reverse detrimental changes could facilitate expansion and differentiation of a normal phenotype. In addition, it would overcome a major barrier to translation of this approach into clinical practice.

11. Alternative cell sources

Researchers in tissue engineering continue to search for cell sources with regenerative potential. Those working on the urinary tract have considered, besides the urologic organs, candidate cells from non-urologic tissue and stem cells.

Buccal mucosa is a non-urologic source that has been cultured successfully for use in urethroplasty in particular [69–72]. Keratinocytes from various sources, including the back of minipigs, foreskin in a rabbit model, and oral mucosa, have been used as alternate cells for urologic tissue engineering [73–75].

Research in stem cell technology is expanding and holds much promise for providing an endless source of non-diseased material for the generation of specific cells or tissue required for replacement and reconstruction. Two important features of stem cells are self-renewal and potency [76]. Self-renewal is the ability to divide and produce unlimited numbers of additional cells with identical differentiation status. Potency is the inherent ability to differentiate into specialized cells. The degree to which these features are present defines different stem cell types. Stem cells can be pluripotent or multipotent. Pluripotent stem cells can be embryonic (EPS) in origin or induced (IPS) by reprogramming of differentiated cells. EPS have unlimited self-renewal and division potential, and can differentiate into endoderm, ectoderm, or mesoderm lineages. Thomson et al [77] were the first to isolate human EPS; since then, numerous such cell lines have been developed particularly in the USA [76]. However, moral and ethical concerns remain over the use of human EPS. Another concern is their pluripotency and the associated inherent malignant potential due to uncontrolled expansion.

Multipotent stem cells are adult stem cells (ASCs) or committed progenitor cells (CPCs). ASCs are capable of self-renewal; CPCs can differentiate but cannot self-renew. ASCs and CPCs are found in all tissues and are a potential reservoir in local tissues and an alternative cell source for tissue replacement. ASCs can be from blood, skeletal muscle, testis, umbilical cord, or placenta [76].

Much work is now being carried out in exploring the potential of a putative stem cell population within the adipose stromal environment (ADSC). Subcutaneous white and brown adipose tissues have the potential to benefit metabolism by improving glucose homeostasis compared to visceral white adipose tissue, which has detrimental effects. ADSCs can also be reprogrammed to IPS cells more efficiently than other cell types. In addition, adipose tissue is abundant, has angiogenic and immunomodulatory properties, and can be harvested with minimal invasion compared to cells from bone marrow [78–81].

An important ASC subtype is stromal mesenchymal stem cells (MSCs). MSCs are derived from connective tissue of most organs; they are fibroblast-like in culture and can differentiate into connective tissue. MSCs derived from bone marrow can generate skeletal muscle with potential to repair damaged muscle in vivo [82].

CPCs can be identified in bladder biopsies and have been isolated from the urine of patients. This noninvasive method of isolating CPCs is attractive and could well inform future research protocols [83]. This is controversial, as most urine-derived cells are not long-lived urothelium in origin and are thought to probably originate from renal tubules. Another major issue is how to differentiate them into a functional tissue, and this has not yet been demonstrated.

Tissue-engineered constructs in the future are likely to incorporate stem cells as alternative cell sources for tissue engineering, but additional research is required before this becomes widespread accepted practice.

12. Conclusions

The treatment options for end-stage bladder disease include bladder augmentation and substitution with alternatives such as bowel. However, the bowel is not structurally or functionally suited to exposure to urine; therefore, it is not surprising that there is a higher risk of infection and of calculi due to mucus production, along with metabolic and cellular changes.

Regenerative medicine offers hope via tissue engineering to develop alternative urothelium-based solutions with or without the help of scaffolds. Researchers around the globe are looking at engineering a neobladder using different strategies, some more complicated than others. Attempts to regenerate urothelial and smooth muscle cells in vitro and then implant the onto a scaffold in an in vivo bioreactor has met with limited success and long-term results have not been encouraging. In terms of strategy, composite cystoplasty remains promising as the concept is based on adapting a clinically effective solution with just the urothelium to be tissue engineered. We will need to further optimise culture systems while rejuvenating or reversing the phenotype before clinical translation to humans.

Conflicts of interest

The author has nothing to disclose.

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