Curr Bladder Dysfunct Rep (2015) 10:443–448
DOI 10.1007/s11884-015-0327-2

Future Perspectives in Bladder Tissue Engineering

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Published online: 16 September 2015

Abstract Substantial clinical need persists for improved autologous tissues to augment or replace the urinary bladder, and research has begun to address this using tissue engineering techniques. The implantation of both tissue scaffolds, which allow for native bladder tissue ingrowth, and autologous bladder grafts created from in-vitro cellularization of such scaffolds have been tested clinically; however, successful outcomes in both scenarios have been challenged by insufficient vascularity resulting from large graft sizes, which subsequently limits tissue ingrowth and leads to central graft ischemia. Consequently, recent research has focused on developing better methods to produce scaffolds with increased tissue ingrowth and vascularity. This review provides an update on bladder tissue engineering and outlines the challenges that remain to clinical implementation.

This article is part of the Topical Collection on *Reconstructed Bladder Function & Dysfunction*

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Keywords Urinary bladder · Regenerative urology · Tissue engineering · Stem cells · Autologous graft

Introduction

There is a substantial clinical need for autologous tissue to augment or replace the urinary bladder, a complex organ with a multi-layered structure. The urothelium serves as an impenetrable barrier providing urine containment [1]. The lamina propria houses vasculature that provides essential oxygen and nutrients to the urothelium, while anchoring it to the bladder muscle [2]. The detrusor muscle facilitates low pressure urine storage and coordinated contractions to expel urine [3]. Individually, the layers serve specific roles; however, taken together, they are all necessary for the unique and essential functions of the bladder.

Tissue engineering and regenerative medicine have led to autologous bladder tissue creation. This occurs with either the implantation of scaffolds with subsequent ingrowth of surrounding bladder tissue layers, or via in-vitro generation of a bladder tissue graft using a cell-seeded scaffold [4•]. Limited vascularity after implantation into the bladder remains a major challenge, thereby making viability of the regenerated tissue unreliable [5]. Furthermore, the ability of diseased bladder cells to successfully colonize scaffolds is often impaired, and the safety of their use in grafts may be limited by certain disease states, such as spina bifida or bladder cancer [6]. These factors highlight the potential for utilizing stem cells in creating autologous bladder tissues.

The field of regenerative urology is steadily growing, and with it, progress is being made in developing autologous bladder tissues. The need to replace or augment the bladder persists as the organ is removed for malignancy or fails secondary to inflammation, neurologic disease, injury, or aging. The

ability of the bladder to regenerate from autologous cells will decidedly advance care for many urologic patients. The following review provides an update on bladder tissue engineering and the current challenges that limit clinical implementation.

Engineering Autologous Bladder Tissue Remains a Complex Challenge

There remains a substantial need for partial or whole bladder replacement. Within the pediatric population, conditions such as bladder exstrophy, bladder outlet obstruction, and neurogenic bladder associated with spina bifida result in severe bladder dysfunction. In contrast, adult bladders are prone to anatomic and functional loss due to surgery, radiation, inflammation, repeated infection, denervation, or trauma. As the population ages, the prevalence of detrusor underactivity increases as well [7]. Bladder augmentation or replacement with bowel is often utilized when bladder capacity and compliance are poor; however, the use of bowel leads to metabolic, infectious, stone-related, and potential oncologic complications [8]. Therefore, there is a substantial clinical need for better autologous bladder tissue that augments or replaces the bladder without such unwanted consequences.

The urothelium is comprised of a multi-layered syncytium of epithelial cells that is impervious to urine. Urothelium displays surface properties which reduce the risk of infection, and it regenerates to cover areas denuded from various insults [9]. Its regenerative capability stems from a muti-layered structure, whereby the most mature cells lining the bladder are shed and replaced by younger cells in the deeper layers. This stratification also facilitates maintenance of the blood-urine barrier as the bladder stretches during filling [1]. Additionally, the urothelium contains cells which transduce bladder sensations, including irritation, distention, and pain [10]. Furthermore, various neurotrophins expressed in the urothelium are intricately involved in bladder function [11–13]. Overall, the viability of the urothelium depends upon the passive diffusion of oxygen and nutrients from the richly vascular lamina propria beneath it.

The bulk of the bladder is comprised of the lamina propria and detrusor muscle. The lamina propria anchors the urothelium to the detrusor and provides innervation [2]. The detrusor muscle has a rich intramural and superficial vascular network [3]. Both layers must be compliant as the bladder fills, in order to facilitate low pressure storage of urine. The detrusor muscle then generates an adequate and synchronous contraction to effectively expel urine from the bladder.

If the bladder undergoes abnormal development or is subject to a substantial insult that impairs its tissue quality, its regrowth and regeneration can be impaired. One example is the fibrotic, contracted bladder that can result from repeated large transurethral bladder tumor resections, which likely results from cauterization of the underlying vascular supply. Similarly, pelvic radiation therapy, chronic inflammation, and recurrent urinary tract infections can cause fibrosis that decreases capacity and compliance, possibly resulting in a minimally functional end-stage bladder [14, 15]. In all these conditions, irritative voiding symptoms develop likely secondary to neural inflammation and altered neurotrophin levels [11]. Such neurologic effects probably interfere with coordinated bladder function, as well. Furthermore, one recent study showed impaired growth of bladder exstrophy smooth muscle and urothelial cells into a collagen scaffold. As such, the potential utilization of such scaffolds in diseased bladders remains uncertain [16]. This finding suggests a potential role for stem cells in re-cellularizing autologous bladder tissue in such disease states.

A variety of scaffold materials exists for bladder implantation or graft creation. These are comprised of both naturally derived and synthetic materials, either separately or in combination. In - vivo placement of scaffolds with surrounding tissue ingrowth and the in - vitro seeding of grafts have been studied for some time [17, 18]. The use of a scaffold implanted for the ingrowth of surrounding tissue requires a healthy bladder and is variably size-limited across species [19•]. The use of growth factors, such as vascular endothelial growth factor (VEGF) or basic fibroblast growth factor, compared to seeding scaffolds with stem cells or other supporting autologous cells, may address this limitation [4•]. Such factors may enable the growth of bladder tissue further into scaffold edges and result in implants with clinically useful sizes.

Similarly, the in-vitro initiation and development of cell layers onto grafts for later implantation may provide a viable solution. However, developing a robust multi-layered bladder graft is challenging because cell culture is often diffusion-limited to no more than 2 mm in depth [20]. Furthermore, one review summarized that vascular infiltration of tissues occurs at a rate of less than 1 mm daily and therefore can take up to 2 weeks to complete in a 3-mm thick tissue [21]. To date, the lack of vascularity upon implantation in such grafts has often resulted in decreased viability further from the graft-tissue interface [22•]. As such, the optimal solution to engineering a bladder graft requires further research.

The Current State of Autologous Bladder Tissue Engineering

Bladder Tissue Scaffolds

Scaffolds for use in bladder tissue engineering are either derived from natural sources through decellularization and processing, or are created from synthetic materials using various fabrication techniques. Hybrid scaffolds that

incorporate elements of both have been developed with the aim of optimizing mechanical properties, degradation rates, intrinsic cytokine content, and cellular interactions [23]. One study utilized a porcine bladder acellular matrix and silk fibroin hybrid for scaffold implantation and observed successful cellularization [24]. Furthermore, the scaffold appeared to generate a multi-layered bladder wall consisting of urothelium, lamina propria, and detrusor muscle that resembled native control bladder; however, stone formation on the graft material and central graft perforation without vascular development also occurred [24]. These limitations highlight current challenges facing bladder tissue engineering research.

Altering the physical characteristics of a scaffold is one method of decreasing bladder stone formation and increasing cell adhesion. Two earlier studies highlight this by demonstrating how nanoscale surface characteristics reduce stone formation and improve urothelial cell coverage and smooth muscle ingrowth [25, 26]. More recently, a three-dimensional synthetic bi-material scaffold comprised of electrospun poly3-caprolactone and poly-L-lactic acid was created with specific nanometer - scale surface features that facilitated the growth of organized urothelial cells in - vitro [27]. Material engineering and nanotechnology fabrication techniques can serve as a means of incorporating important characteristics into scaffolds.

Scaffold content and structure are additional important considerations. To address insufficient vascularity, Jiang et al. utilized a 2 cm×3 cm scaffold of rabbit bladder acellular matrix and impregnated it with polylacticcoglycolic acid nanoparticles to provide prolonged VEGF release [28•]. This resulted in increased microvessel density and reduced scaffold contraction and collagen content. Additionally, the scaffold was mechanically tested and demonstrated physical characteristics similar to the native bladder [28•]. This supports previous research in which incorporation of VEGF resulted in improved scaffold muscularity, angiogenesis, and innervation [29]. Similarly, integrating adipose-derived stem cells into a scaffold resulted in the development of mature urothelium, nerve, and bladder muscle [30]. This was in contrast to ingrowth of urothelium only in the scaffold alone group. These findings highlight the potential for utilizing molecular elements or cells to improve bladder tissue substitutes.

The mechanical characteristics and physiologic activity of the bladder tissue that forms on scaffolds is a crucial element of their design. Specifically, material selection for scaffold creation contributes to the mechanical properties of the graft prior to being resorbed. Also, the structure and organization of tissues that replace a scaffold determine the overall characteristics and functionality of the new bladder segment [31]. The finding that urinary diversion impairs the development of fetal bladders suggests a role for mechanical loading from filling and voiding in the organized formation of bladder tissues [32]. Similarly, when a scaffold was utilized for augmentation cystoplasty in a canine model, animals that underwent prolonged catheter decompression of the bladder developed disorganized and fibrotic tissue at the scaffold site [33]. In contrast, limiting catheterization duration resulted in the growth of robust urothelium backed by increased vascularity, neuronal processes, and smooth muscle. This suggests that physiologic loading of bladder scaffolds is necessary for mature bladder tissue development.

Bladder Tissue Grafts

In contrast to the use of bladder tissue scaffolds, the in-vitro creation of a bladder graft may provide an optimal solution for limited tissue development. This is particularly true in cases where diseased bladder cannot be used due to the risk of cancer or advanced bladder decompensation and fibrosis [16, 34, 35]. The ability to develop an in-vitro layer of urothelium onto grafts has been possible for some time [18]. Subsequent work has shown that urothelium can be generated from pluripotent stem cells, eliminating issues related to creating bladder tissue from a diseased source [36]. More recently, stratified grafts containing both urothelial and smooth muscle layers have been created and utilized clinically [22•, 37]. However, outcomes in these trials were limited due to implant contracture and central (distal) graft necrosis. Contraction and necrosis remain major challenges to autologous bladder graft success.

Insufficient vascularity in bladder grafts with subsequent implant contraction or perforation is a challenge similar to that witnessed in scaffolds with limited peripheral tissue ingrowth. Since cell culture techniques rely largely upon diffusion to maintain cell viability and support growth, the ability to form a sufficiently thick multi-layered tissue can be limited. Surgically, attempts have been made at improving graft perfusion after implantation by utilizing an omental overlay, but these interventions have been limited in their success by central graft degradation and perforation [22•]. This highlights the need to increase vascularity within autologous grafts in - vitro. However, the cell culture environment may not provide sufficient stimulus for functional vessel formation, which emphasizes improving graft vascularity as a priority area for research.

An excellent study utilizing partial cystectomy and bladder segment transplantation assessed the roles of graft and host vasculature and cellular regenerative capabilities. Osborn et al. demonstrated that native graft vessels remain intact centrally, while the proximal edges of a 5-mm bladder graft contained host vessel ingrowth and chimeric vessels [38•]. This suggests that inosculation is possible with a graft containing vasculature, and this facilitates graft survival. Furthermore, the results of this study showed that host stem cells do not contribute substantially to angiogenesis, but rather, they replace the donor urothelium [38•]. These findings further suggest that

the incorporation of vessels into bladder grafts developed ex vivo may contribute to their successful utilization.

Similar to scaffold implantation, the choice of material for in-vitro bladder graft creation is also an important factor. Materials should provide an ideal environment for the growth of urothelium, lamina propria, and smooth muscle [4•]. The substrate of the graft must also serve as a barrier to urine in order to protect the viability of the underlying lamina propria and smooth muscle layers until the urothelial barrier matures [5, 19•]. The material must display appropriate mechanical properties and degrade at the proper rate to optimize its integration into the bladder and minimize the risk of stone formation on the graft [39, 40]. As a bladder graft is developed in - vitro and ideally comprised of three layers, expediting vascularity appears to be the most pressing issue to ensure tissue viability and minimize the risk of urine leakage.

Similar to scaffolds, material selection for graft creation contributes to mechanical properties of the tissue segment prior to being resorbed. Moreover, the structure of the newly grown tissue determines the overall physiologic characteristics of the implanted bladder segment [31]. Trials with human bladder graft placement and omental overlay have resulted in clinically insignificant changes in bladder pressure and volume, as well as a number of complications related to graft viability and omental flap placement [22•, 37]. Bladder decompression with continued catheter drainage was maintained postoperatively in these patients for 3 weeks, followed by bladder cycling (filling and emptying). With the resolution of graft viability issues, further research is needed to investigate the mechanical loading of the augmented bladder for optimization of the development of normal bladder architecture.

Perspective on the Future of Autologous Bladder Tissue Engineering

The successful utilization of bladder scaffolds and bladder grafts is challenged by vascularity, which dictates the extent and rate of native bladder ingrowth and transplanted tissue survival. Most successful bladder scaffold experiments reported utilizing smaller implants since larger segments fail to facilitate viable multi-layered bladder tissue development [4•]. Similarly, urine leakage and graft contraction occur with implantation of large bladder grafts. Expediting vascular development within scaffolds using angiogenic growth factors or multipotent cell seeding could provide solutions for successful clinical implementation.

Since diffusion can support the ingrowth of bladder tissue at scaffold edges and sustain the viability of peripheral graft tissue, an alternative form of implantation could be considered to maximize contact of the graft with the native bladder edges. Otherwise, the use of non-omental vascularized structures, such as demucosalized bowel and stomach segments, may warrant consideration due to their proven ability to sustain urothelial cells in - vivo [41, 42]. Alternatively, a staged approach at augmentation that utilizes the incorporation of smaller viable graft segments may be explored. The field of autologous bladder tissue engineering remains a prime area for research with notable opportunities for innovation.

Aside from vascularity and viability, the challenge of creating structurally normal bladder tissue must be recognized. The ability to regenerate urothelium onto scaffolds and grafts has been proven for some time since urothelial cells are relatively easily replicated [18, 36]. Similarly, smooth muscle has been visualized in small scaffolds in - vivo, as well as certain grafts in - vitro [22•, 26]. A recent study demonstrated that graft preparation in - vitro using a type 1 collagen substrate seeded with bladder mesenchymal cells, when exposed to urine, produced urothelium with physiologic pseudostratification and cellular polarity [43]. Furthermore, the seeded bladder mesenchymal cells migrated to the urothelial layer, suggesting interaction between the two cell types. The authors of this study theorized that exposure to the molecular contents of urine provided an additional stimulus to promote more physiologic urothelial development. Further research in this area, and others such as formation of a robust lamina propria, is warranted.

Other components of bladder tissue critically important to optimize are its mechanical properties and physiologic functions. Normally, bladder volume increases in a highly compliant manner without a significant rise in pressure. A recent review suggested that bladder acellular matrix may be the optimal substrate for scaffolds and tissue grafts due to its retention of endogenous growth factors and its inherent biomechanical properties [19•]. Still, regardless of which autologous tissue substrate is utilized, to achieve optimal cellularization and adequate substrate degradation remains the biggest challenge. Furthermore, the exact role of how a mechanical stimulus remodels both bladder scaffolds and grafts remains unclear. Based upon observations made during both in-utero bladder development and in clinical trials of autologous bladder augmentation, insufficient mechanical stimulus from fill and empty cycles is associated with tissue fibrosis and poor compliance [22•, 32, 33, 37]. Investigation into the optimal approach to each of these factors is needed.

Even as in-vivo and in-vitro generation of urothelial and smooth muscle layers becomes routine, developing bladder tissue with a functional lamina propria may prove elusive. This remains an important consideration because of the vascularity and innervation that this tissue layer provides [2]. Based upon the current field of research, lamina propria creation seems to be less of a challenge with scaffold use because tissue ingrowth can result in a normally contracting and complete 3-layer bladder, especially when endogenous factors are utilized or exogenous ones are added; however, with larger scaffold sizes, this has not necessarily held true [4•, 19•, 28•,

29]. Since bi-layer materials have shown promise in graft creation, a tri-layer approach could be reasonable if cells incorporated in the center remain viable in a diffusion-limited cell culture environment [24, 27, 43]. Reliably obtaining a robust lamina propria remains a significant challenge.

Considerations Moving Forward

With both stem cells and autologous bladder augments being tested in clinical trials, the field of regenerative urology is expanding. A substantial clinical need exists for autologous bladder tissue to either augment or replace the native bladder. It is essential that high-quality tissue is produced with normal structure, mechanical properties, and function. The ideal manner of achieving this has yet to be determined, but numerous options remain. The best substrate for scaffold or graft formation has yet to be identified, but a number of promising possibilities have been discovered. For individuals with a diseased bladder, stem cells provide an excellent option for graft seeding when autologous urothelium is unsafe or damaged. Additional optimization of autologous bladder tissue through improved vascularity is an area of active research, and the use of cytokines and stem cells will hopefully facilitate this. Lastly, clarifying the role of mechanical stimuli in improving scaffold or graft function is a key consideration that should not be underestimated.

Taken together, once autologous bladder tissue generation has been improved sufficiently, creation of an entirely autologous bladder represents the next visible frontier on the horizon. Each of the aforementioned challenges will be pertinent to this venture and potentially compounded in complexity once a three-dimensional structure is involved. Other new technologies and techniques will likely prove crucial as capabilities in autologous tissue development progress. Considering the use of grafts with vascular pedicles and tissue expanders in plastic and reconstructive surgery, could researchers could grow a bladder elsewhere within the body and subsequently transplant it into its orthotopic position? The possibilities remain plentiful for the future of regenerative urology.

Conclusions

Great progress has been made in bladder tissue engineering. However, clinical success with grafts remains inconsistent. Many challenges remain, and these are largely related to improving tissue vascularity and viability. Once these hurdles are passed, identifying the optimal means of bladder cycling for physiologic tissue organization is a remaining obstacle. The fields of materials engineering, stem cells, and nanotechnology hold great potential for further advancement in bladder tissue engineering.

Acknowledgments Support provided in part from NIH R01 HD059859, NIH R21 HD078820, the Department of Veterans Affairs Rehabilitation Research and Development Service (VA Merit B7225R), and the Cleveland Clinic.

Compliance with Ethics Guidelines

Conflict of Interest B.C. Gill declares that he has no conflicts of interest. According to M.S. Demaser, "Although I have grants from industry and a pending patent in the general field of testing regenerative agents for incontinence recovery, none is directly related to the material presented in this paper." To quote C.J. Chermansky, "Although I am Co-investigator on 2 grants and a site PI on a multi-center industry trial, none is directly related to the material presented in this paper."

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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