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Review Article

Challenges and Perspectives in Male Anterior Urethra Reconstruction Using Tissue Engineering

Christophe Caneparo¹, Stéphane Chabaud^{1*} and Stéphane Bolduc^{1,2}

¹Centre LOEX de l'Université Laval, Génie tissulaire et régénération, centre de recherche FRQS du CHU de Québec, Axe Médecine Régénératrice, Quebec City, QC, Canada

²Department of Surgery, Laval University, Canada

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ABSTRACT

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Corresponding author:

Stéphane Bolduc,

Research Center in Experimental Organonogenesis / LOEX, Quebec Hospital - Laval University Research Center, 1401 18th Street, Quebec City, Quebec, G1J 1Z4, Canada, Email:stephane.bolduc@fmed.ulaval.ca

Repair or replacement of the male anterior urethra remains a challenge in 2019 especially in the case of patients presenting severe defects due to the paucity of tissues available for reconstructive surgeries. Nowadays, surgeons mainly use buccal mucosa, which can come with complications not only at the donor site but also at the graft site. This option is sometimes invasive in the case of long defects when large pieces of tissue are needed. Tissue engineering is an emerging field in regenerative medicine and offers promising avenue. It allows reconstruction of large amount of autologous tissues using small biopsies. Several strategies of tissue engineering use biomaterials, which can cause adverse effects. A new option appears with the use of the "self-assembly" protocol to reconstruct tissues using only the cells of the patients and avoiding the use of biomaterials. Human-derived tubular structures were produced by this technique and present mechanical and functional properties compatible with grafting. Moreover, these tissues can be endothelialized to prevent graft ischemia. New challenges in tissue engineering is to differentiate the cells needed for the reconstruction from induced pluripotent stem cells derived from patient blood cells, and to avoid the use of animal serum for cell culture.

INTRODUCTION

The function of the urinary tract is to produce (kidneys), convey (ureters), store (bladder) and finally excrete (urethra) the urine. Part of the reproductive function is also performed through urethra and neighboring tissues. The male urethra is divided in two parts: the posterior urethra, consisting in prostatic and membranous urethra, and the anterior urethra, consisting in the bulbar, pendulous or penile urethra, and fossa navicularis. Except for the fossa navicularis section, the urethra is lined by a pseudostratified epithelium known as urothelium. The urothelium consists in three parts: the basal layer containing cells with a great potential of division, the basal, progenitors and stem cells; several intermediate layers containing intermediate cells with some potential of division, depending of their level of differentiation; finally the superficial layer containing flattened cells without potential for division and terminally differentiated (sometimes binucleated), the umbrella cells. In the fossa navicularis, the epithelium is stratified squamous, roughly similar to the oral mucosa. Around the urethra, the urethra, a spongy tissue surrounding/supporting the urethra, is found the corpus spongiosum, a spongy tissue surrounding/supporting the urethra, the corpus spongiosum, a spongy tissue surrounding/supporting the urethra, sponget tissue surrounding/supporting the urethra, the terminal terminal



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and the tunica albuginea, an elastin rich fibrous envelope. A thicker and more resistant version of tunica also envelops the two corpora cavernosa, the erectile tissue.

CLINICAL CONDITIONS

Male anterior urethra can be affected by several congenital and/or acquired anomalies, which could require surgical reconstruction to restore a normal genitourinary function. Hypospadias, for pediatric patients, and urethral strictures, for adult patients, are amongst the most common urethral pathologies in need of such reconstruction.

Hypospadias is the most frequent malformation of the penis forming three-quarters of all congenital penile anomalies [1]: about 1 in 200 to 250 newborn males is affected [2,3]. Recent studies have reported an increasing prevalence of hypospadias [4-8]. It represents a defect in the tubularization of the urethral plate leading to a shorter urethra, resulting in an inadequate position of the meatus below the tip of the glans. The opening can be positioned anywhere along the ventral side of the penis, if the meatus is located to the dorsal side, the anomaly is called epispadias. The severity of hypospadias is determined depending on where the meatus is located: minor defect if the opening is close to the glans or severe defect if the meatus is close to the scrotum. Severe defects often require surgery [9-11]. Due to its high prevalence and its impact, hypospadias represent an important health issue [12]. The cost of treatments could also be high. Indeed, patients affected by the most severe form of the congenital anomaly often suffer from postoperative complications such as complete dehiscence, urethral/meatal stenosis or urethra-cutaneous fistulae, and then require subsequent surgeries [13].

The other frequent urethral disorder is the stricture. A stricture is the narrowing of the anterior urethra. Narrowing affecting the posterior urethra is called stenosis [14]. Male urethral stricture disease most commonly results from injury, instrumentation, noninfectious inflammatory conditions of the urethra, hypospadias surgery and finally sexually transmitted disease [15]. Urethral strictures result in more than 5,000 inpatient visits yearly in the USA. Yearly office visits for urethral stricture numbered almost 5 million between 1992 and 2000 [16]. The total cost of urethral stricture diseases in USA in 2010 was around \$300 million. Yearly cost for health care expenditures is increased by more than \$6,000 per individual following urethral stricture diagnosis [17]. Patients with urethral stricture also appear to have a high rate of urinary tract infection (41%) and incontinence (11%) [18].

CURRENT SURGICAL REPLACEMENT OPTIONS

Repairing or replacing the urethra can be done using a wide variety of tissues, such as skin grafts (including genital and extragenital skin flaps), tunica vaginalis (around a testicle), and bladder, lingual or oral mucosa [19-24]. This latter is the current gold standard. However, many complications are encountered such as pain, numbness, submucosal scarring, salivary duct obstruction and injury [25,26]. The first use of the oral mucosa as urethral replacement has been done by Kirill Sapezhko, a surgeon from the Russian Empire, in 1890 [27]. This pioneer technique was also reported by Graham Humby in UK in 1941 [28] and "rediscovered" in 1992 by Bürger in Germany [29] and Dessanti in Italy [30]. However, in comparison to autologous urethral tissue, all of these substitutes present limitations, which can lead to complications [31-33]. Furthermore, despite improvement of the harvesting technique, donor sites remain limited in the amount of tissue that can be harvested. This lack of sufficient amount of tissue to graft can be problematic in the case of long defects. Moreover, in the case of post-operative complications, the surgeon will not harvest this mucosa twice from the same site, limiting the surgical options. To overcome these difficulties, alternative methods for urethral reconstruction have been explored.

TISSUE ENGINEERING STRATEGIES

Current alternative strategies consist in the use of tissue engineering for urologic regenerative medicine [34,35]. Because of a lack of ideal tissue to reconstruct the urethra, many tissue engineering strategies have been attempted. The ideal engineered tissue to graft should be biocompatible, functional and support capillary and/or vascular networks. Some techniques are using biomaterials such as synthetic or natural polymers and acellular matrices [36], which have many advantages such as the possibility to dictate the macroscopic form of the graft and the ready-to-use format.

Synthetic biomaterials such as poly (L-lactide) (PLLA) [37] and poly(lactide-co-glycolide) (PLGA) [38] have been tested. Such materials give the possibility to make biocompatible 3D- organs at a low cost, with controlled mechanical properties and degradation rates. But it also allows obtaining rapid and



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reproducible results with low risk of biologic contaminants. However, synthetic biomaterial degradation could be problematic due to the presence of their hydrolytic degradation products released in the body. Furthermore, synthetic biomaterials do not provide an adequate environment for an optimal epithelial cell differentiation and organization, preventing the graft to fulfill its biologic functions [39].

In order to circumvent the drawbacks of using synthetic materials, several groups have used natural biomaterials such as type-I collagen [40] or silk [41]. The results remain poor with a 40% failure rate with collagen structures [42] which could be diminished to 10% when using low fiber density collagen graft [43].

Decellularized extracellular matrices have also been used, such as Small Intestine Submucosa (SIS), pericardium, Bladder Submucosa (BSM) and acellular corpus spongiosum matrix, for urethral replacement [44-46]. By decellularizing it, it is expected that mechanical properties will stay intact and biochemical environment remains identical to the living tissue. Despite the good results obtained, significant amounts of residual DNA have been detected, representing an immune risk for patients [47]. Indeed, if techniques used are too aggressive, extracellular matrix properties could be lost. The challenge is therefore to discard all immunogenic factors without losing of beneficial factors. Furthermore, vascularization was shown to be problematic since a lack of nutriments and oxygen can lead to ischemia, necrosis, fibrosis and transplant failure.

The role of the cells inside the biomaterials, whatever their nature, remains essential especially to repair challenging conditions. Numerous types of cells have been used in urethral tissue engineering [48]. Urethral substitution were attempted by using acellular [41,42,49] or cellularized matrices [34,50-55]. A major concern about the use of acellular matrices is that urothelial regeneration (migration from host tissues) in acellular graft is limited to 0.5 cm in length, which compromises success in more complex cases, such as long strictures [56]. Tissueengineered matrices containing autologous cells, in addition to extracellular matrix, seem more promising. Using this method, a large autologous-cell graft could be produced from a small biopsy, with the ability to grow in vivo without rejection. Moreover, studies have reported that stem cells can be obtained from urine [57,58], making this approach even more attractive. Recently, Induced Pluripotent Stem Cells (iPS) also raised the attention of several groups to generate large amount of cells needed for the reconstruction [59-61]. Despite significant progress in the urethral tissue engineering field, there is very few published clinical trials [62]. However, the clinical trials conducted so far present good results in a limited number of patients with long-segment and/or complex stricture disease [20,63-66]. Many different techniques have been used in those trials, therefore, no consensus, can be established. The other question being debated is: should we test the tissue engineering options in first line cases that usually present fewer complications and offer a large volume of patients, or should we test it in severe and challenging cases where actual options are not optimal. If we test the tissue engineering models in those complex cases, it will face a greater challenge and it will be more difficult to establish it as an effective alternative.

SELF-ASSEMBLY TECHNIQUE

Because of a lack of ideal tissue to reconstruct the urethra, many tissue engineering strategies have been described. Nevertheless, despite the positive results of these biomaterials [67-69], weakness are persisting [47,70] leaving space for new alternatives. A novel way to produce reconstructed tissues has been discovered at the end of the twentieth century. Indeed, in the presence of ascorbic acid, human fibroblasts cultivated in vitro produce extracellular matrix such as collagen, which allows within a few days, establishment of a three-dimensional tissuelike construct that can be manipulated [71,72]. This method called "self-assembly technique" has been further used to reconstruct blood vessels in 1998 [73]. Through the years, many tissues have been reconstructed using this technique such as bladder [74-76], hypodermis [77], urethra [78,79], cornea [80] and skin, with pathological or physiological diseases: psoriasis [81], scleroderma [82], hypertrophic scarring [83] and melanoma [84]. Severely burned patients are already treated by grafting autologous skin made with this technique [85]. This method is explained in (Figure 1A) for the flat model (reconstructed urethral patch) and (Figure 1B) for the tubularized model (tubular reconstructed urethra). This technique allows the reconstruction of urologic tissues presenting histological features similar to the native ones (Figure 2A).









Figure 1: Self-Assembly technique. A. To produce a urethral patch. B. To produce a tubular urethral model. Ascorbate is used during cell culture. Production of the stroma takes 1 month and differentiation of the urothelial cells another month.



constructed urethra stained by the Masson's trichrome protocol. Extracellular matrix is in blue and cells in pink/purple. Differentiation of the urothelium with basal, intermediate and superficial cells can be seen. B.6-cm Tubular substitutes in the bioreactor chamber. The mechanical strength could be appreciated. C. Tubular structure is prepared for the graft.



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Because of its expensiveness, precise technical skill requirements and long culture time, this technique remains minimally used in the tissue-engineering field. However, recent research introducing the use of lysophosphatidic acid, allows reduction in cell culture time by increasing the amount of collagen deposited and consequently the stromal thickness, with improved mechanical properties resulting in a faster formation of a manipulable tissue. Also, by modifying the initial protocol, the production of tissue has been rendered relatively inexpensive and easy to perform while recreating a more physiological organization of the stroma, especially the distribution of the cells throughout the extracellular matrix [86, 87].

Clinical studies on urethroplasty showed a success rate of 81% when using buccal mucosa [88], not including the problems at the harvesting site, and 84.2% when using tissue engineered oral mucosa [89]. So, it could be expected that urethral substitute reconstructed using tissue engineering with organspecific cells should be an improvement. It is therefore possible, from a patient's biopsy, to build stromal sheets, which could be rolled to create an autologous 3D urethra [78]. Mechanical characteristics of these models are roughly similar to the native tissue. Autologous epithelial cells can be seeded and cultured to maturity in the tube by using a dynamic flow in a bioreactor to mimic the in vivo tissue architecture [79]. Cells are therefore mechanically stimulated (Figure 2B,C) and tissues are strengthen by inducing a realignment of collagen fibers, as previously shown [90,91]. These reconstructed urological tissues were shown to express uroplakins and zonula occludens-1 in the epithelial superficial layer, which are essential for tissue function to prevent urine leakage. As vascularization of the graft is an important obstacle to avoid graft ischemia, the potential for adding endothelial cells has been tested in these models, in order to prevascularized them [86,92]. Tissues were successfully grafted on the back of mice. To further reduce the size of the biopsy needed to reconstruct the tissue, protocols to preserve stem cells during the expansion of the biopsy-extracted urothelial cells has been developed [93]. And in order to improve the quality of the urothelial differentiation, the use of organ-specific cells has also been recently done [94].

However, the use of animal models is required before human implantation to validate the model and avoid unnecessary complications for patients. Rabbits have similar urethral organization compared to the human and have already been chosen to test urethral reconstruction based on collagen gels [40]. Producing a tube, which can be mechanically compared to the human model, is the main objective. However, due to differences in the protocol to produce rabbit stromal tissue [38], it could be preferable to use human stromal tissue, presenting a very weak immunogenic potential [95], instead of rabbit stromal cells. The goal is to cure a human condition with humanspecific engineered tissues, not to develop a rabbit model. Therefore, introducing modifications to the techniques to produce the human tissue could be a drawback with regard to the regulatory agencies. We must keep our production protocol as simple as possible and we need a uniform technique (same for animal implantation then for the human application) to build our case for the regulatory authorities. Urothelial cells autologously extracted from rabbits will be seeded on these human stroma and tubes, vascularized or not, and will be maturated under dynamic culture conditions to improve stromal and urothelial characteristics before in situ implantation in rabbits. These animal implantations will pave the way to the first clinical trial in human's patients.

PERSPECTIVES

The reconstruction by tissue engineering of a human-derived organ-specific autologous urethra has been an actual challenge due to the complexity of the specification: combining good mechanical properties to adequate differentiation of the urothelium, but it has been achieved using the self-assembly method, i.e. without the use of biomaterials. Several new challenges appear such as the use of urothelial, mesenchymal and endothelial cells differentiated from induced pluripotent stem cells derived from blood cells. It would allow circumventing the need to take a biopsy with potential comorbidities, but also to avoid the issue when facing the complete absence of specific cells in a given patient, or presence of inadequate cells to harvest [96]. Another challenge in the future years should be to exclude the use of serum into cell culture. Serum could not only induce loss of reproducibility in some experiments but also be a source of animal contaminants and also raise ethical concerns [97].







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