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Evolution in Tissue Engineering for the Lower Urinary Tract

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1. Introduction

The organism is a complex machinery, which is composed of different systems acting in synergy to maintain the homeostasis and the integrity of the human body. When one of these systems cannot provide its regulating function, the quality of life and the survival of the organism are imperiled. The purpose of the urinary tract is to ensure equilibrium and consistency of the blood components. Thus, it maintains the osmotic tension, the acido-basic and hydro-electrolytic balance. The urine is elaborated from the blood medium and then evacuated out of the body. It consists of metabolic, nitrogenous and exogenous wastes, which makes it a toxic biological liquid for cells and structural proteins.

The human urinary system is composed of two sections; the upper urinary tract which includes the kidneys and the ureters; and the lower urinary tact that includes the bladder and the urethra. The bladder is a watertight and compliant reservoir in charge of urine storage before its evacuation via the urethra under sphincter control. In order to preserve the integrity of the upper urinary system, the maximum capacity of the bladder is approximately 500 mL. This eliminates possible reflux which could damage the renal function. There are two main differences between the female and male urinary tracts; the presence of the prostate and the length of the urethra. As showed in figure 1, the length of the female urethra is around 3 to 4 centimeters compared to 20 centimeters for the male.

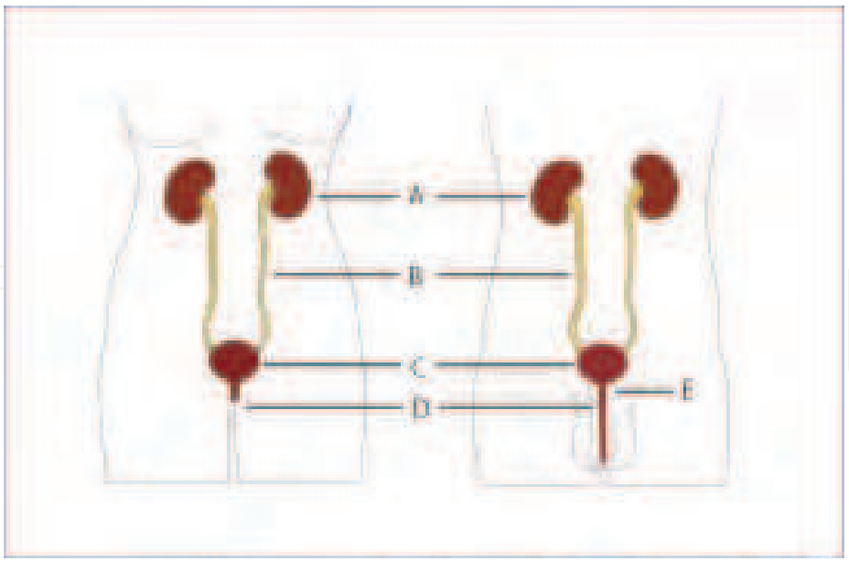

Fig. 1. Schematic representation of the urinary tract in woman and man. A) Kidneys. B) Ureters. C) Bladder. D) Urethra. E) Prostate.

As shown in figure 2, for the bladder and the urethra, the bladder mucosa is underlined with a unique and highly specialized epithelium, known as urothelium, which rests on a basal lamina. Underneath, the lamina propria is made of fibroblasts, microcapillaries and fibers of collagen mostly of type I and III. The detrusor muscle is formed by smooth muscle cells with interfascicular connective tissue containing fibers of collagen I and III. Finally, the presence of a serosa completes the bladder wall. The presence of collagen type I, III and elastin largely determine the mechanical properties of the bladder wall (McCarthy et al 2003). For the urethra, the mucosa is similar to the bladder but a smooth muscle cells layer replaces the detrusor and the serosa is not present.

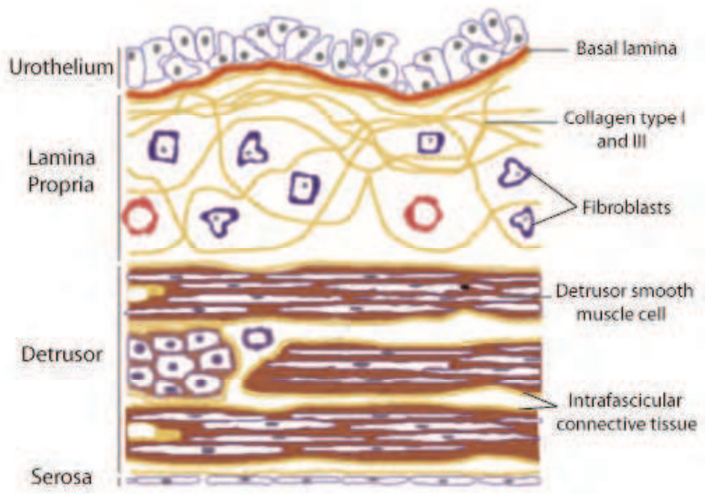

Fig. 2. Histological representation of the bladder structure of the mucosa.

All bladder layers have an important role, however we will focus our attention to the urothelium since it is the tissue which is directly in contact with the urine. In order to limit the passage of water and ions between urine and the bloodstream, the luminal superficial cell layer is composed of so called umbrella cells, which are derived from the basal urothelial cells precursors (Bolland & Southgate 2008). Umbrella cells are characterized by the presence of a specialized asymmetric apical membrane (AUM), which is an arrangement of uroplakin proteins by specific interactions, to form a protective barrier on the apical surface (Veranic et al. 2004). It is important to note that uroplakin organization is therefore a specific differentiation marker of the urothelium (Sun 2006).

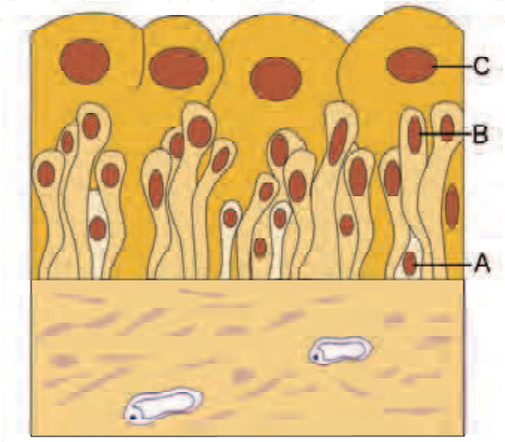

Fig. 3. Schematic representation of the pseudo-stratified urothelium. A) Basal cell. B) Intermediate cell. C) Superficial cell also known as umbrella cell.

Several conditions like congenital malformations, cancer, traumatisms and stricture can cause problems that will require surgical treatments to replace the injured section. To rectify those low urinary tract problems, partial or total reconstruction might be needed. In urology, the need for autologous tissues with properties similar to the native tissues is an important limitation. However, in organ regeneration, tissue engineering recently made great advances. It regroups biological, clinical and engineer disciplines that elaborate physiologically functional tissues similar to the native one found *in vivo*. Contrarily to the beginning of tissue engineering, cellular and molecular organizations are strongly considered nowadays. It is the reason why this discipline needs different matrices and host cells to reproduce a substitute conform to the original organ. Different types of substitutes, going from non-urologic tissues to different types of biomaterials, have been developed and used to guide *in vivo* regeneration on the injured site. The host cells migration from the extremity toward the center of the graft, consistently lead to post-surgical reorganization of the implanted substitute. This is why all current research studies head toward the fact that seeding cells *in vitro* on a matrix support will lead to better post-surgical results. This chapter will describe the tissue engineering position in urologic reconstruction and explore its evolution with the different types of strategies used.

2. Native Non-Urologic Tissues: the Beginning of Tissue Engineering

2.1 Bladder

In view of the necessity to maintain the compliance of pathological bladders, several types of tissues have been used unsuccessfully. The skin, the omentum, the stomach, the pericardium, representing native substitutes (Draper & Stark 1956, Goldstein et al. 1967, Andretto et al. 1981, Nguyen & Mitchell 1991), but also silicone, polyvinyl and Teflon, for the alloplastic materials (Kudish 1957, Bogash et al. 1960, Kelami et al. 1970), but they quickly encountered significant complications. The absence of biocompatibility, vascularization and the immunogenicity of some of these substitutes, as well as the direct contact with the toxic urine, lead mainly to the formation of fibrosis and contraction of the graft (Burbige & Hensle 1986, Vemulakonda et al. 2008). In parallel, the substitution of the bladder by the means of various intestinal segments has been practiced more successfully since the beginning of the eighties (Tariel et al. 2006). While taking into account the limits previously observed, this technique called enterocystoplasty is the clinical treatment currently used.

Unlike the first substitutes described, the intestinal segment allows improvement in compliance. Moreover, it has the advantage of being richly vascularized, preventing necrosis, inflammation, and thus, the contraction of the graft. However, like the first native substitutes, the mucous membrane of the intestinal segment has absorption properties and electrolytic secretion which are specific to it. Thus, the incorporation of these various native tissues involves metabolic imbalances specific to each one, as well as the formation of mucus and urolithiases, which are significant morbidity factors (Mundy & Nurse 1992, McDougal 1992). Its addition to the biochemical profiles of mucous membranes used, the bad contact with the toxic urine leads to risks of neoplasic transformation of the native substitutes (Woodhams et al. 2001, Ali-El-Dein et al. 2002), as well as structural deterioration of alloplastic substitutes (Cross et al. 2003). The incorporation of intestinal segments previously de-epithelialized was attempted to avoid mucus production and potential reabsorption within an animal model but it lead to fibrosis, and then, the contraction of the graft because of the urine being in direct contact with the underlying submucosa and extracellular matrix (Hafez et al. 2005).

2.2 Urethra

Several non-urologic tissues have also been tried for urethral reconstruction: ureter in 1909 (Schmieden 1909), saphenous vein in 1910 (Tuffier 1910) and appendix in 1911 (Lexer 1911). The skin was first used in 1914 (Nové-Josserand 1914) as a split thickness graft rolled into a tube. The results were not as expected because of the contracture of the graft, so it was abandoned until 1941 (Humby 1941) when a full thickness skin graft from the genital region was reported. In 1948 (Mc Indoe 1948), a split thickness graft technique was used. The patient had to wear a dilator for 6 months before the intervention as a tissue expander to obtain the harvest skin. The same year, the skin sample was used for urethral reconstruction (Vyas et al 1987, Young & Benjamin 1948).

The use of skin graft for urethroplasty is of two types, split or full thickness. For the full thickness skin graft, various donor sites like penile foreskin, supraclavicular skin, inner

aspect of the upper arm, abdominal wall, buttock and thigh have been used for their different characteristics (Devine & Horton 1977, Hanna 1983, Hendren & Crooks 1980, Shapiro 1984). At the moment, the most commonly used remains the penile skin and foreskin as a flap, because they are more pliable and less thicker than the other types of sampling (Vyas et al 1987). Also the absence of hair in those two sites reduces the probabilities of complications. In general, good results have been obtained with the use of skin for urethroplasty. However, post-surgical complications still occurs. At the harvesting site, both type of skin graft can leads to poor cosmetic wound healing. Moreover, both types of skin grafts can lead to poor cosmetic wounds at the harvesting site. At the reconstruction site, complications like recurrent strictures or fistulas are found. Also, *Balanitis Xerotica Obliterans*, a genital skin inflammatory disease, can occur. Finally, hair growth from hair bearing harvest site, with or without concretion and graft contracture has been observed. The use of split thickness skin graft has shown a reduced tendency to contract, but it comes with a lack of adequate resiliency and strength (Markiewicz et al 2007). Actually, penile and foreskin skin flaps are considered the gold standard for urethroplasty, but when they are not available, due to use of that skin in a prior intervention, circumcision, presence of scar tissues or because of a long urethral defect, other tissues are needed for urethral reconstruction (Theodorescu et al 1998).

The first report using oral mucosa for urethroplasty was in 1941 (Humby 1941). It was not used again until 1992 (Burger et al 1992) where it was reintroduced as another free graft material for urethral reconstruction like penile skin. Two sites for oral mucosa are most commonly used for harvesting: the buccal and labial mucosa of the lower lip. If there is a lack of available genital skin, oral mucosa is a good replacement graft material because it is easily harvested, non-air bearing and in abundant supply even for adult cases. It has furthermore interesting properties like elasticity, abundant submucosal vascularity, thick mucosal layer and it is tolerant to air and liquid exposures (Caldamone et al 1998). Also, this avoids cosmetic disadvantages and consequences caused by the use of genital skin harvested from the penis (Barbagli & Lazzeri 2006). The morbidity associated with oral mucosa is pain, discomfort, limited range jaw opening, neurosensory defect and salivary flow modification. Those complications are temporary, going from 1 to several months depending on the harvesting site. The post-surgical complications found after urethral reconstruction are still fistula formation, stricture and meatal stenosis (Barbagli & Lazzeri 2006, Markiewicz et al 2008, Markiewicz et al 2007). A study compared oral mucosa with penile skin for urethroplasty and obtained a similar overall success rate with no significant difference and in conclusion, they were declared both excellent materials for urethral reconstruction (Alsikafi NF 2005).

Bladder mucosa for instance was first introduced in 1947 for urethroplasty, but significant complication rates delayed its clinical use until 1981 (Coleman et al 1981), where it was associated with good results in the case of a deficit of penile skin. It gained an immediate popularity with the clinicians because of its structural similarity with the urethra. However, due to the lower abdominal incision required to access the harvest site, the procedure is technically complex, it often results in poor esthetics and delay in wound healing occurs at the donor site. The principal complications found at the reconstructed site are mucosal prolapse and meatal stenosis (Markiewicz et al 2007, Vyas et al 1987). To overcome the

complications found with the use of bladder mucosa, a combination of preputial or penile skin with bladder mucosa have been used (Duffy et al 1988). A reduction of the meatal stenosis was observed, but no other follow-up studies on this combination were found afterward.

For female urethral reconstruction, vaginal mucosa have been used (Brannan 1951). Urethral stricture or fistula in female are not as frequent and it is related to continence problems (McKinney 1979). The principal advantages of vaginal mucosa are the same as oral mucosa. It is easily available, hairless, elastic, naturally wet and it avoids any cosmetic wound problems. Good results have been obtained and limited complications have been reported post-surgically (Tsivian & Sidi 2006).

Other non urologic tissues have been studied for urethroplasty for specific situations when the gold standard is not available. In 1967, tunica vaginalis found around the testicule was first reported in an experimental study for urethral reconstruction (Ariyoshi 1967). It was recommended because it is a readily available source, simple to harvest and use. Other advantages such as a lack of hair follicles, a good tensile and physical property resulting in better sutures holding and a very good vascularization were reported. Over the years, it has been used in experimental studies for surgical techniques in urethroplasty (Calado et al 2005, Khoury et al 1989, Talja et al 1987) and in general, good results were observed. In 1998, a study compared an onlay flap with a tube flap performed on a New Zealand rabbit model with a control group. The New Zealand rabbit is the standard animal model used for urethral reconstruction. Their conclusion was that an onlay flap was more suitable for long term reconstruction, because it was further vascularized than the tube flap (Theodorescu et al 1998). In 2009, another study combined tunica vaginalis with preputial island flap, on a rabbit model for 12 weeks to overcome the complications found with the use of tunica vaginalis alone (Leslie et al 2009). Even with that combination, fistula and urethral diverticulum occurred. Clinically, the use of tunica vaginalis was evaluated in two studies with different conclusions. One found the use of tunica vaginalis useful mainly in difficult cases as long as there is no external exposure (Snow & Cartwright 1992) and the other found no advantage to its use as an onlay flap in proximal urethral repair due to its high stricture rate (Joseph & Perez 1999). The use of tunica vaginalis is now for coverage purposes, to protect the reconstructed area. Another tissue was used for an experimental study, the use of autologous vein graft on the rabbit model without strong conclusions (Kahveci et al 1995). More recently, colonic mucosa has been suggested for the urethral replacement in case of long and complex stricture. A dog model has been studied and it was also used for human urethral reconstruction with relatively good results. The principal complications at the harvest site were not described but at the reconstruction site: meatal stenosis, bulbar or bulbomembranous urethral stenosis and proximal anastomotic site stricture were observed with low complication rate (Xu et al 2004, Xu et al 2009, Xu et al 2003).

3. Acellular Tissue Matrices

The acellular matrix are obtained from native tissues, decellularized and sterilized. Thus, they are naturally cytocompatible and biodegradable, which represents a major asset as a substitution support. Various methods are used to carry out their preparation (Rosario et al.

2008), but the general outline presents a mechanical delamination of the tissue followed by an enzymatic treatment, an hypotonic solution to destroy cell residues and finally, the sterilization with ethylene oxide or peracetic acid. This kind of substitute has the advantage of presenting mechanical and biochemical properties, comparable to the native tissue. Indeed, the processing procedure allows preserving the three-dimensional environment, favorable to the cell infiltration, migration and proliferation within the substitute (Sutherland et al. 1996). The infiltrating cells then could have the suitable organization and differentiation to produce their own matrix during the resorption of the support, but this one is not in conformity with the physiological architecture. Indeed, these models cannot be identical since the mechanical and chemical treatment for the acellularization and sterilization, inevitably deteriorate the composition and organization of the matrix proteins (Brown et al. 2002). Let's remember that proteins are prone to unfolding which can be caused by temperature increase, other denaturing biophysical elements, like the pH, and biochemical agents, like urea. Thus, elasticity and mechanical resistance are decreased, which might not allow size variations during the bladder cycles of filling and emptying. Nevertheless, the aptitude to support the neovascularization is preserved (Lantz et al. 1993, Kajbafzadeh et al. 2007), at least the presence of endothelial cells precursors was observed post-graft, which is kinetically supported by the frequent use of a 100 μm thickness graft, instead of the full thickness.

3.1 Bladder

The small intestinal submucosa (SIS) was the first marketed model. After acellularization, a matrix mainly made up of collagen (I, II, VI), glycoaminoglycans, proteoglycans and glycoproteins like fibronectin was obtained. The combination of these various structural proteins allowed the storage of multiple growth factors, thus making them available to the surrounding cells. The heparan sulfate proteoglycans (HSPG), fibroblast growth factor 2 (FGF2), transforming growth factor beta (TGF-β), and the vascular endothelial growth factor (VEGF), essential to promote the graft neovascularization, were detected by immunofluorescence (Hurst & Bonner 2001), but in vivo regeneration showed only limited results in the rat and the dog models (Zhang et al. 2006). Indeed, the bladder capacity was improved partially even after 9 months post-operatively, however rapid deaths of one fourth (1/4) of the dogs due to perforation within the SIS had to be accounted for. To explain such results, an hypothesis was put forward by the same team at the sight of in vitro results, after urothelial and smooth muscle cells seeding (Zhang et al. 2000). Cells showed a good SIS adherence, but their organization and differentiation were not observed; which is however essential to preserve the graft size and to restore the functionality of the pathological bladder. A certain cytotoxicity was noticed concerning the SIS matrix, in addition to its immunogenic nature. Indeed, a series of postoperative inflammatory reactions (Ho et al. 2004) preceded the in vitro detection of porcine DNA residues on the commercially available SIS, by specific chromatin staining (Feil et al. 2006). These last reports made on this model, confirmed it as an unfavorable substitute for vesical reconstruction.

Recently, it was reported that the specific use of the organ to be regenerated is the most appropriate for the cellular organization and functionality (Sievert & Tanagho 2000). Thus, the bladder acellular matrix graft (BAMG) constitutes a logical substitute for the cell

development. It was studied on various animal models, after being characterized in vitro. Under physiological conditions, the bladder matrix is made up of structure proteins, proteoglycans and glycoaminoglycans, able to trap and store the growth factors. Particularly, the matrix network constitutes a biochemical and spatial database, specific to the organ-source, thus informing the cells about the type of organization to be adopted. The bladder matrix therefore seems to be a substitute of choice, but after acellularization and sterilization treatments, the BAMG obtained displayed a diminished composition of elastin, laminin and fibronectin (Farhat et al. 2008). Collagen I and IV are preserved while the collagen III, one of the most abundant isoforms under the physiological conditions, seems to be degraded. The molecular architecture deterioration of the BAMG could potentially decrease its regenerating capacities. However, the dynamic culture of smooth muscle cells, within the BAMG, displayed encouraging results on the collagen I, III and IV synthesis (Farhat & Yeger 2008).

Beforehand, stretching forces were applied, which generated a growing interest to determine the impact of mechanical stress on the cell-cell and cell-matrix interactions (Baskin et al. 1993, Adam et al. 2004). However, this type of stress does not correspond to the physiological forces applied to the bladder. Dr. Farhat's team developed a bioreactor able to apply hydrostatic pressure waves, thus imitating the bladder filling and emptying (Wallis et al. 2008). Briefly, the seeded porcine BAMG, which is seeded with urothelial and smooth muscle cells, is placed between two chambers. One of which will be used to apply a pressure, gradually increasing on the presenting cell surface. At the end of four hours, the pressure reaches 10 cm H_2O, intravesical pressure at the fœtal stage, to fall abruptly in a few seconds to zero, to act like a voiding. The mRNA expression of matrix proteins increased significantly under mechanical stimulation and the mRNA expression of differentiation markers presented encouraging results. Indeed, mRNA coding for uroplakin II seemed to increase, which is promising for obtaining a watertight model and avoiding deterioration by the urine. This mode of dynamic preconditioning constitutes a promising alternative to counterbalance the deterioration attributable to the acellularized tissue preparation. Unfortunately in this study, only the gene expression was studied, which does not constitute sufficient information, since the protein functionality depends on its complete synthesis, its good folding and its appropriate localization, among other things. This bioreactor design is ideal to elaborate a bladder substitute under similar physiological conditions, but it still needs optimization. For example, the chamber design does not allow uniform distribution of the pressure waves on the tissue in place, but the evolution of this work is followed with attention. Meanwhile, studies in static mode continue and present relatively favorable results after implantation in a porcine model (Merguerian et al. 2000); in spite of the recurring complications observed, such as calcification formation and graft retraction. Urothelial and smooth muscle cells infiltration was noted but their localization remained limited to the graft periphery, and their organization was incomplete. The already raised hypothesis is that acellularization treatment affects the bladder matrix architecture. The BAMG is then obtained with a too high porosity to retain the growth factors and to promote the cell propagation (Farhat et al. 2003). In static culture conditions, porosity reduction was observed by hyaluronic acid incorporation during rehydratation phase of lyophilized BAMG (Cartwright et al. 2006). Hyaluronic acid is an essential glycoaminoglycan, known to support cell proliferation and migration in the extracellular

matrix. The study's authors also claimed to make a barrier against urine with this method, but let us remember that urine pH and ionic forces have denaturing properties for nonspecific urothelium proteins.

BAMG and SIS are also limited by their xenogenic source. The rejection of this type of substitute is a serious possibility in sight of epitope presence, specific to the organ-source. The Gal oligosaccharide was found in the porcine SIS extracellular matrix, and that, even after the various preparation treatments (Badylak & Gilbert 2008). This antigen is present on the cell surface of many species, except human and a category of monkeys, which poses problems of immunological recognition post-graft. In the same way, it is not possible to eliminate any risk of viral contamination. To get around these undesirable characteristics, in addition to the differences inherent to the acellularized matrices preparation and their source (Kropp et al. 2004), the idea of constructing neobladders in a reproducible way was made possible by the use of synthetic polymers.

3.2 Urethra

The use of acellular matrix relies on the fact that it could have an effect with its environment because the extracellular matrix could influence the biological behavior of the cells (Farhat et al 2008) and lead to a better integration of the graft. In the literature, many acellular tissue matrices are called collagen matrix, for example, the bladder acellular matrix graft (BAMG). The principal advantages of using a BAMG matrix, as the other types of acellular matrices for urethral reconstruction is that it is an "off the shelf " material that eliminates all other surgery required for harvesting. It may also shorten the operative time, since it is easily prepared in large quantity in laboratory and can be stored until its use (Chen et al 1999, De Filippo et al 2002). Many studies have been done on the use of this type of material alone or with cells. A study was first done using xenogenic BAMG from a porcine source on the rabbit model for 6 months with an onlay fashion graft. Their goal was to evaluate if the use of BAMG would be suitable for a urethral reconstruction on an animal model (Chen et al 1999). Their results have demonstrated that it was appropriate since there was no evidence of infection, graft rejection, fistula or stone formation. In 2003, a study was done with an allogenic BAMG coming from cadaveric human bladder tissue on 28 adult patients (El-Kassaby et al 2003). A follow-up of 36 to 48 months was made and good results were observed, only 4 strictures occurred. Finally, another study was done with xenogenic BAMG in a tubular form from a porcine source, on a rabbit model for 1 month but reported opposite results (Dorin et al 2008). Their goal was to determine if there was a maximum limit for urethral defect in which an acellular matrix would be sufficient for replacement. The results showed that an unseeded tubularized BAMG may be successful when used for repair of a relatively small urethral defect of less than 0,5 centimeter. It is also well documented that re-anastomosis in those small urethral defects is very successful, so it would seem to limit the potential value of acellular tubularized matrix graft. The study suggested that for a urethral defect over 1 centimeter, the need to seed cells on acellular matrix may be necessary to allow the growth of healthy urethral tissue and avoid stricture formation and other complications. It also showed the discrepancy observed in the last decade from the literature regarding the success of acellular matrices for tissue regeneration on small animals and the failure on large animals and humans (Dorin et al 2008).

Due to these problems, studies began to report the importance of seeding cells on BAMG for urethral reconstruction. Those studies were done using xenogenic and allogenic BAMG seeded with cells and they were compared to unseeded BAMG as control. A first study using a porcine xenogenic BAMG in a tubular form was seeded with urothelial and smooth muscle cells extracted from New Zealand rabbit's bladders and implanted on a rabbit model for 6 months. The results showed that BAMG seeded with cells from normal bladder tissue can be used for a tubularized replacement, whereas BAMG without cells lead to poor tissue formation and stricture (De Filippo et al 2002). Another study, with an allogenic model consisting of BAMG seeded with foreskin epithelial cells from New Zealand rabbit's was performed on the same kind of animal for 6 months. The results were similar to the previous study, the BAMG seeded with epidermal cells obtained better outcomes than BAMG alone. Also, the research team claimed that the use of epidermal cells seemed adequate to replace urothelial cells (Fu et al 2007). The problem was that after 6 months, the epidermal cells kept their structural form rather than acquiring the transitional structure known to the urothelial cells. An additional study was done with rabbit allogenic BAMG. This time, the team seeded rabbit's oral keratinocytes and the study was done on rabbit model for a duration of 6 months. Their results showed that oral keratinocytes have a good biocompatibility with BAMG. It confirmed the prior results that seeded BAMG are superior to unseeded BAMG (Li et al 2008a, Li et al 2008b) and the oral keratinocytes kept their structure of stratified squamous epithelium rather than the transitional form of the urothelial cells, even after 6 months. In conclusion, cell seeded matrices seem to allow tissue regeneration across the defect and the maintenance of a non-obstructive outflow from the bladder; it confirms the need to incorporate cells.

For urethral reconstruction, other tissues were studied as acellular matrix graft. Small intestinal submucosa (SIS) was first suggested because it was shown to promote tissue specific regeneration in a variety of organs. SIS is composed of a collagen meshwork with various intrinsic growth factors, cytokines, structural proteins, glycoproteins and proteoglycans that may assist in cell migration as well as cell growth and differentiation during the regenerative process (Colvert et al 2002). The advantages of using this type of tissue are similar to BAMG matrices. A pilot study was done using xenogenic SIS, from a porcine source on a rabbit model for 3 months. Their goal was to evaluate the suitability of the SIS onlay patch in urethroplasty (Kropp et al 1998) and its feasibility, but it was a short-term study and not further mentioned. Also, the use of homologous acellular urethra was suggested for urethral reconstruction. The advantage of using an acellular urethra is that the extracellular matrix comes from the same place, so the various intrinsic growth factors; cytokines, structural proteins, glycoproteins and proteoglycans are identical. It allows a single-stage intervention with a tissue that has the same form and dimension. A research team studied the use of a homologous acellular urethra matrix for urethroplasty on a rabbit model for 8 months (Sievert et al 2000). Their goal was to evaluate urethral replacement by a free homologous graft of acellular urethral matrix on a rabbit model. They made a complete study of the different parameters on the take of the graft. Their results demonstrated a functional and histological regeneration. Another team did a similar study. They also used an allogenic acellular urethral matrix on a rabbit model. Their goal was the same but their implantation was for 6 months (Hu et al 2008). The results obtained were not convincing but they were similar. Also, an acellular aortic matrix was used for urethral reconstruction. The

goal of this research team was to create an experimental model of urethral defect and to repair it using allogenic acellular aortic grafts as urethral substitutes (Parnigotto et al 2000). The advantages of using an aortic acellular matrix are the initial tubular structure with suitable dimensions and it is directly applicable. The surgeon can decide the length of the defect as well as the size of the urethra to be reconstructed. In general, they obtained good results, however, it was not further mentioned.

4. Synthetic polymers

Synthetic polymers are thermoplastic materials made of biodegradable composites that can be tailored to the patient's organ size. Mechanical and structural properties such as the size of the pores of these biomaterials can be controlled and their biological degradation is done by hydrolysis (Freed et al. 1994). It can also be produce in big quantity at a relatively low cost.

4.1 Bladder

The biomaterials synthesis, such as polyglycolic acid (PGA) and polylactic-co-glycolic acid (PLGA), offers a controlled composition after each production. They are authorized by the FDA and generally used for clinical drug encapsulation. This concept to isolate content from external space is particularly attractive for the bladder, since it can be used for the elaboration of a urinary storage bag. In vivo studies were performed where dogs were subjected to partial bladder replacement, using a PGA/PLGA scaffold, with cultured urothelial cells seeded on the luminal surface and smooth muscle cell on the exterior surface (Oberpenning et al. 1999). A functional evaluation was performed for 11 months which displayed an increased capacity to retain urine. This study demonstrated, most of all, that successful reconstitution of an organ was promoted when using cultured cells in vitro. The construct without cell seeding led to contraction and shrinkage already seen at the first post-graft month. Although histological results seemed to have a normal architecture, the study did not specify if the in vivo cellular organization was present just at the periphery or on the whole synthetic graft. Indeed, the migration of surrounding cells has not been frequently observed in the middle of the graft, where inosculation with host's vasculature takes more time in this area. Nanobiotechnology is well-positioned to influence synthetic scaffold optimization, but at cellular scale, the coating with a 3T3 mouse fibroblast as a feeder layer for urothelial cells already demonstrated an increased proliferation (Drewa et al. 2006).

In the same way as for the canine model, Atala's team continued clinical tests on candidate patients for cystoplasty suffering from high pressure bladders or pathological non-compliant bladder, by using the same scaffold wrapped with omentum but using a mixed of collagen and PGA (Atala et al. 2006). This study provided two key elements. First, the culture of urothelial and smooth muscle cells in a three-dimensional environment was feasible. Second, a nutritional support by the highly vascularized omentum wrapped around the synthetic scaffold provided positive effects on success. The young age of the patients theoretically promotes in vivo regeneration, but after 5 years, only one of the seven patients seemed to have significantly increased bladder compliance. In spite of everything, the biopsies obtained months after implantation showed a good organization of urothelial and smooth muscle cells, but the authors did not explain this paradox with the post-

operative urodynamic results. Generally, the disadvantages of these polymers lie in the bad cicatrization with the host's tissue, as well as in the absence of biological recognition. However, on this last point, the nanobiotechnologies have the capacity to improve them by integrating cellular recognition domains in their structure, such as RGD peptide (Cook et al. 1997).

Harrington's review defines the discipline as the creation of objects or a surface whose unique functions are a direct result of their nanoscale dimension/organization (Harrington et al. 2008). The beginning of engineered synthetic matrices focused on the three-dimensional and porosity aspect, but to imitate the cellular environment with a more efficient approach, composites at a lower than 100nm were used to elaborate scaffolds since extracellular matrix proteins are nano-dimensional. To direct the cellular phenotype, the nanofibers composites are designed like collagen I and III fibrils, the most abundant isoforms of the native bladder. The conventional methods are based on a similar concept of adhesive protein interactions, such as non-covalent links, to form supramolecular polymer. Two synthesis techniques are commonly used. The « bottom-up » synthesis consists of triggered nanomolecules which self-assemble into a molecular object, like the micelles formation from individually charged lipids. In this case, the aggregate dimension is pre-determined through the incorporation of features with specific adhesive properties. The « top-down » approach is a technique in which untreated material is specifically degraded to result in a nanoscale patterned surface, as with lithographic techniques. Therefore, it appears evident that the new generation of biomaterials must manage the cells in their native language. Indeed, the incorporation of cytokines and the growth factor signaling could influence the cell behavior and promote controlled tissue regeneration. The challenge of this discipline is to precisely copy the extracellular matrix proteins architecture, to inform the cells of their physiological organization. In the bladder tissue engineering field, some studies of nanoscale materials were successfully completed (Baker et al. 2006, Han et al. 2006, McManus et al. 2007). Bladder smooth muscle cells adhesion and enhancement in elastin and collagen deposition were measured with the application of nano-structured PLGA/polyether-urethane scaffold (Pattison et al. 2007). The same study showed that synthetic composites are not yet spared from urine degradation, which led to post-graft bladder leaks in a rat model. Moreover, the clinical application of this cell-seeded construct still remains expensive.

4.2 Urethra

Many synthetic polymers have been studied over the years, alone or with cells to add more support to the material. The principal advantage using synthetic polymer is that it decreases the inflammatory reaction and other adverse effects, it is biodegradable and it eliminates the risk of hair growth or stone encrustation on hairs in the urethral lumen that can be found with the hair baring skin. Because it is an artificial material, it eliminates complications related to harvesting. Furthermore, it is possible to play on the different proprieties of the polymer like its porosity, the length and the diameter required for urethroplasty (Olsen et al 1992). At the end of the 70's, some alloplastics were studied for urethral reconstruction: Silicone, Dracon, Gore-Tex and Teflon were used without great success on dog and human (Court et al 1971, Court et al 1973, Gilbaugh et al 1969, Hakky 1977). They were unsuccessful and these materials could bring post-surgical complications like fistula and periurethral

haematomas (Dreikorn et al 1979). With the improvement of technology, new synthetic polymers appeared and were studied for urethroplasty. In 1992, a tube formed of polyhydroxybutyric acid (PHB) coated with polyglycolic acid (PGA) on the inside was tested for urethroplasty on a dog model for 12 months. After 8 months, the polymer had completely disappeared and the lumen of the urethra was somewhat wider than normal and no complications were reported (Olsen et al 1992). These results were interesting but they were only based on macroscopic and histological examinations, no further studies were made after. In 1997, a group suggested to use guide implant for short urethral gaps. They made a pilot study on a new biodegradable and highly biocompatible polymer, the hyaluronan benzyl ester (Hyaff-11) on a rabbit model for a month. The implant guide was compared to two control groups; one using a silicone guide and a negative control. The principal advantages of the Hyaff-11 resides in its inertness, its possibility of producing devices with variable rates of biodegradation, its favorable profile of toxicity and biocompatibility, its properties of protecting delicate tissues and its potential for wound healing. In this pilot study, no complications were observed except at the suture site where microfistulas appeared. On histology, the use of Hyaff-11 seemed to promote the proliferation of urothelial cells in cuboidal form in opposition to the silicone guide, where the urothelial cells were in regression and in a squamous epithelium (Italiano et al 1997). No further studies were made with this material for urethroplasty.

More recently, a research team studied the use of a polyglycolic acid (PGA) stent coated with a polylactic-co-glycolic acid (PLGA) solution and seeded with bovine chondrocytes (Amiel et al 2001). They compared the model's reaction *in vitro*, in static condition, in a bioreactor and *in vivo*. It was implanted subcutaneously in a nude-mouse, for 4 and 10 weeks. The ability to implant chondrocytes in the genitourinary tract has been demonstrated in the literature for the treatment of vesicourethral reflux (Atala et al 1993, Atala et al 1994, Diamond & Caldamone 1999), urinary incontinence (Atala et al 1994) and penile prosthesis (Yoo et al 1998, Yoo et al 1999). Their final goal was to use autologous chondrocytes to obtain a biocompatible model. Generally, good results were obtained but further long-term studies have to be made on animal models. Also, the effect of toxic urine on the model has to be evaluated. Another team studied the use of synthetic polymer with cells on a rabbit model for 24 weeks (Fu et al 2009), a poly L-polylactic acid (PLLA) polymer stent seeded with autologous urothelial cells from the urethra. They used PLLA because it has a satisfactory mechanical strength in cross-section and moderate longitudinal flexibility, making it easy to adapt its expansion so that it can ensure support for the urethra and avoid collapse. The structure of the polymer also provides a good environment for cell adhesion. Good results were obtained. After 12 weeks, the polymer was degraded and after 24 weeks, the cells were implanted successfully, survived and regenerated well. However, it remains a short-term study. Like for other acellular tissue matrices, the use of cells in new synthetic polymers helped prevent complications and provided a better repair after urethroplasty. Even with these results, other materials are chosen before them and as one can see, not a lot of studies have been made to support their long-term use in urethral reconstruction.

5. Autologous approach: the self-assembly method

To this day, the integration of previously described substitutes has caused chronic inflammation or graft rejection. That is explain by the degradation of biosynthetic or xenogenous scaffold, that release non-recognized materials. The last few years, the organ reconstruction field knew the emergence of the self-assembly method (Auger et al. 2002), based on the cell capacity to produce their own matrix support. This approach consists of a cell culture in well defined conditions that will construct an autologous scaffold free of exogenous biomaterials. At the Laboratoire d'organogénèse expérimentale (LOEX), the self-assembly method proved its capacities in skin, blood vessel, and cornea regeneration (Pouliot et al. 2002, Tremblay et al. 2005, Germain et al. 1999), for graft or pharmacological studies. This is why our team works to elaborate autologous urological tissues. We investigate the feasibility to reproduce the physiological structure of bladder mucosa and genitourinary tubes. Dermal fibroblasts are interesting cells since, in presence of ascorbic acid (Chepda et al 2001, Davidson et al 1997), they have the ability to synthesize extracellular matrix in an increased way to form a collagen-layer. The autologous sheets could be then superposed or rolled to create a three-dimensional environment for the reorganization of additional cells specific to the reconstructed organ.

5.1 Bladder

The method starts by extracting urothelial, endothelial and smooth muscle cells, from a simple bladder biopsy (1cm by 5cm). This minimally invasive preoperative phase to harvest the three cell types of the bladder, was reported and confirmed good individual cell population purity (Magnan et al. 2006). The endothelialization of any substitute is necessary to promote the survival of the graft. Recently, LOEX laboratory obtained a rapid inosculation with a capillary-like network of reconstructed tissue and the host vasculature (Tremblay PL et al. 2005). Consequently, when cellular sheets were obtained, endothelial cells were added on, one day before they were superimposed, three by three. Urothelial cells were then seeded on top of the three-dimensional construction, and after a proliferation phase, the construction was elevated to the air/liquid interface as showed in figure 4. This technique was inspired from in vitro engineered epidermis, to induce cell maturation, because urothelial and epidermal cells are both epithelial in origin. However, epidermis and urothelium organization are not exactly the same. To generate a more physiological process, the replacement of the air/liquid phase by the dynamic bioreactor culture is currently being considered.

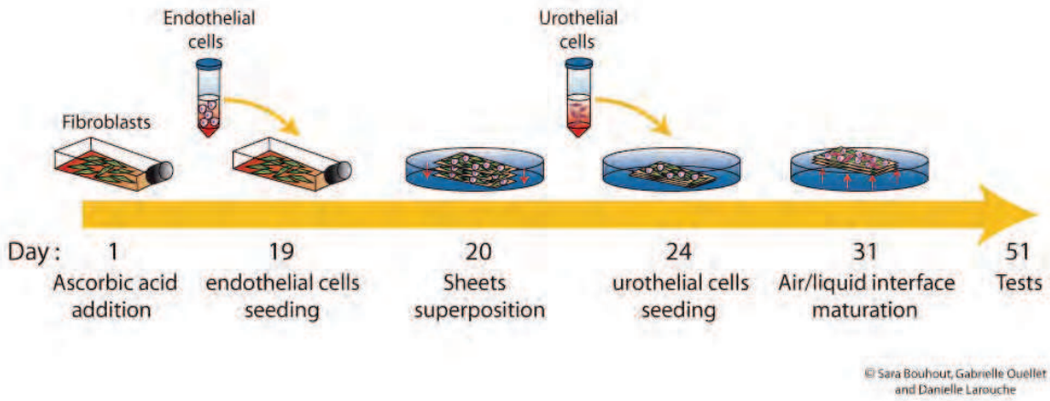

Fig. 4. Schematic representation of the bladder self-assembly approaches.

In the meantime, histological analysis showed a good distribution of the extracellular matrix and the cellular multi-layered morphology appears to be characteristic of normal bladder urothelium. Collagen I and basal membrane, essential to urothelial organization, were identified by immunofluorescence. More interestingly, cytokeratin 8/18 staining confirmed the good distribution of a stratified urothelium on the whole bioengineered support, which is important to avoid urine infiltration. Tissue permeability was evaluated by using Franz's cells, which consists of placing the vesical equivalent between the donor compartment containing radioactive urea and the receiver compartment, from which samples are obtained at different time points to determine permeation profiles. The endothelialized vesical equivalent displayed the same profile as the native bladder, contrarily to a construction of fibroblast only, which showed a fast diffusion of urea. This demonstration proved the necessity of having reconstructed urothelium covering substitutes, in order to avoid urine extravasation and secondarily, in vivo necrosis and fibrosis. Mechanical characteristics of the vesical equivalent seemed to have sufficient resistance to allow suturing. Even if the metalloproteinases released by endothelial cells reduced the vesical equivalent resistance, results remained satisfying because of their superiority compared to the minimal threshold measured from the native bladder (Dahms et al. 1998). Although this model still requires improvements, such as smooth muscle cells incorporation, its autologous origin and its efficiency as a barrier to urea, encourage further optimization in order to make this tissue-engineered equivalent, an ideal bladder replacement.

5.2 Urethra

Our tubular equivalent is build from the same cellular type. As showed in figure 5 and described previously (Magnan et al 2009), mature fibroblasts extracted from the skin are grown in culture medium for 28 days with ascorbic acid to stimulate the production of extracellular matrix. The presence of the extracellular matrix provides the formation of a fibroblasts sheet easy to handle, it enables us to roll it around a mandrel to obtain a tubular form. Then it is grown with another culture medium for 21 days with ascorbic acid, to increase the cohesion between the fibroblasts layers. When we obtain a uniform tube, urothelial cells are then seeded inside the tubular model and placed under perfusion at 15

ml per minute in a bioreactor with culture medium for 7 days with ascorbic acid to promote proliferation and maturation of the urothelial cells.

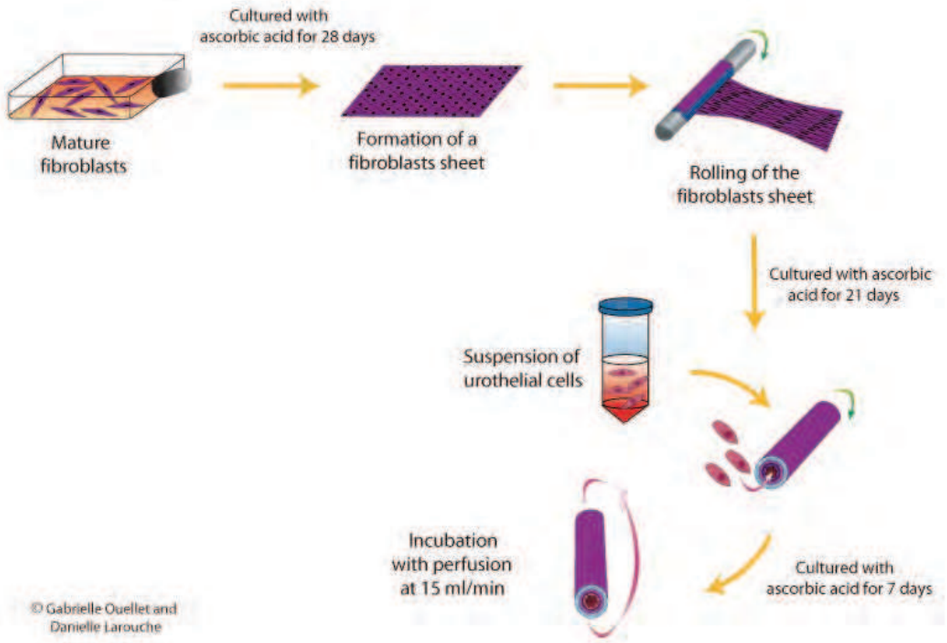

Fig. 5. Schematic representation of the urethral self-assembly approaches.

Macroscopically, the model was uniformly assembled, provided suture resistance and was easily handled. Histologically, we obtained a thick layer of fibroblasts in an abundant extracellular matrix and the urothelium was similar to a native urethra. The urethral model characterization with cytokeratin 8/18 by immunofluorescence and western blot confirmed the presence of a well differentiated and pseudostratified urothelium. The viability test demonstrated that the cells could be reextracted from the tube and reseeded with a 2% mortality rate, which is normally found in cell culture. Finally, the results of the burst pressure tests confirmed a higher resistance when compared to native porcine urethra.

Our autologous urethral equivalent is an innovative model that offers a promising alternative for urethral replacement or reconstruction. Like for alternative tissues, cell extraction will need harvesting from a skin biopsy, but in a smaller amount. To collect the urothelial cells, a bladder wash will be use. But the greatest advantage remains that our model will use the patient's cells with no xenogenic matrix, which should decrease the inflammatory reaction post-surgically.

6. Conclusion

Significant complications of alternative native tissue used for reconstruction raised the need for other types of materials. Tissue engineering had known successes and limits, but those limitations have contributed to move forward the knowledge in urologic tissue regeneration. Naturally derived materials and acellular tissue matrices have the potential advantages of familiar biological architecture. On the other hand, synthetic polymers can be

obtained reproducibly on a large scale with control properties. But the challenge to produce a urologic tissue replacement with a scaffold that demonstrates functional compatibility with the native tissue still remains relevant. With the results obtained from the different studies, the protection against urine toxicity by a watertight construct and the quick vascularization of the urologic substitute are necessary to preserve the cellular growth and avoid graft contraction. The self-assembly approach seems a promising option to obtain an autologous engineered equivalent, endotheliazed and similar to the native tissue. For the future, all tissue engineering disciplines will need to explore new techniques, going from dynamic cell culturing to nanobiotechnologies, to obtain a unique structure and the full functionality of the urologic tissue to replace. We will have to consider the differences between a healthy animal model and the implantation of the substitute into a human patient with an active urologic pathology. This will be a major challenge.

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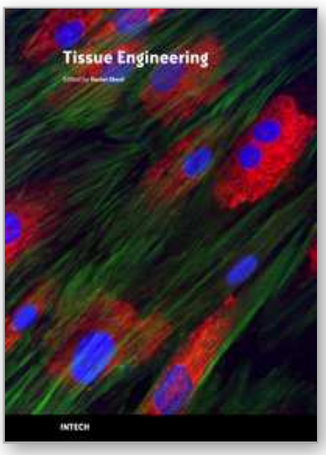

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The Tissue Engineering approach has major advantages over traditional organ transplantation and circumvents the problem of organ shortage. Tissues that closely match the patient's needs can be reconstructed from readily available biopsies and subsequently be implanted with minimal or no immunogenicity. This eventually conquers several limitations encountered in tissue transplantation approaches. This book serves as a good starting point for anyone interested in the application of Tissue Engineering. It offers a colorful mix of topics, which explain the obstacles and possible solutions for TE applications.

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