Abstract

This chapter reviews the use of engineering and biomaterials approaches aimed at improving the capacity and compliance of the diseased urinary bladder. The chapter introduces the normal bladder and describes the clinical background that drives the need for bladder tissue replacement and/or reconstruction. It examines a variety of synthetic and natural matrices developed for different strategies in bladder reconstruction from both historic and contemporary perspectives, and provides a critical review of the results to date, including discussion of the steps needed to realise bladder tissue engineering in patients. Although progress has been made, the need for materials that offer the unique requirements for use in the bladder remains unmet and offers an open opportunity for future research champions in the fields of biomaterials, tissue engineering and regenerative medicine.

Key words: urinary bladder, tissue engineering, enterocystoplasty, bladder augmentation, bladder reconstruction, natural biomaterials, synthetic polymers, acellular matrix.

TISSUE ENGINEERING USING CERAMICS AND POLYMERS

Bladder Tissue Regeneration

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1. Introduction

1.1 The functioning urinary bladder: structure and function relationships.

The urinary bladder is a complex, highly-compliant organ whose functions are to store variable volumes of urine for extended periods of time and to expel urine cyclically by the process of micturition. By retaining urine at safe pressures and preventing retrograde flow, the bladder protects the delicate structure of the kidneys from irreversible tissue damage and organ failure (Thomas, 1997). The remarkable capacity and compliance of the urinary bladder reflects a unique combination of biological, structural and biomechanical properties within the bladder wall. Specifically, the basal tone of the detrusor smooth muscle is responsible for active mechanical tension, while extracellular matrix (ECM) proteins of the lamina propria provide compliance. A further critical component is the urothelium: a specialised transitional epithelium found lining the inner surface of the bladder that forms the urinary barrier and contributes to accommodatory and mechanosensory ("fullness-sensing") functions (Birder et al., 2012). The complexity of the biological:structural:functional relationships in the healthy normal bladder underpins the challenge of developing biomaterials that can be used, either alone or with cells, to patch, augment, reconstruct or even replace a diseased bladder. There is a clear clinical need, but despite the bladder being the focus of many tissue engineering and biomaterials studies, the challenge of finding a functional organ or tissue substitute remains largely unmet.

1.2 Features of the mammalian bladder

The mammalian bladder wall is composed of four concentric layers: an outer single cell serous layer or adventitia that surrounds the three loosely arranged layers of detrusor smooth muscle (FIGURE 1). Within this lies the viscoelastic collagenous connective tissue of the lamina propria supporting an array of cellular structures, including blood vessels, sensory and motor neurons. Type I collagen is the most abundant ECM in the bladder wall, with the conformation and orientation of widely-distributed type III collagen considered implicit to healthy bladder tissue compliance (Chang et al., 1998). The lamina propria is separated from the lumen-bounding urothelium by a capillary-rich proteinaceous basement membrane.

Urothelium is classified as a transitional epithelium as histologically, it is transitional between non-stratified "simple" and stratified squamous type epithelia. The entire urinary tract from the renal pelvis, through the ureters, the bladder and into the proximal urethra is lined by urothelium, but whilst the bladder and lower urinary tract arises embryologically from endoderm, the urothelium of the upper tract is mesodermally-derived (Hicks, 1975, Tanaka et al., 2010, Bock et al., 2014). The development of essentially the same specialised epithelium from two embryological roots is unique and suggests an evolutionary convergent programme of gene selection for urinary barrier function in upper and lower urinary tracts.

Morphologically, urothelium is stratified, with basement membrane-bound basal cells and lumen-facing superficial or "umbrella" cells interposed by intermediate cells that vary from three to seven layers according to the degree of bladder distension. The urothelium is recognised as the least permeable (ie tightest) of all epithelia and maintains the urinary barrier throughout filling-voiding cycles. The major barrier-forming adaptations are located in the large, frequently binucleated superficial cells, with specialised intercellular tight junctions maintaining the paracellular barrier (Acharya et al., 2004, Smith et al., 2015, Varley et al., 2006) and the transcellular barrier provided by multiple thickened plaques of Asymmetric Unit Membrane (AUM) embedded in the outer leaflet of the apical membrane (Hicks, 1965). The AUM plaques are formed through the interactions of four uroplakin ("urothelium-plaque") proteins and are a unique feature of urothelium (Olsburgh et al., 2003, Wu et al., 2009, Yu et al., 1994). AUM plaques are formed in the Golgi apparatus and transported to the apical membrane as fusiform vesicles (Tu et al., 2002), thereby providing

a source of membrane for accommodating the changes in urothelial surface area and helping to maintain a low pressure environment during bladder filling.

For the tissue engineer, barrier function can be assessed in vitro from transepithelial electrical resistance and/or permeability studies (Rubenwolf and Southgate, 2011). In addition, immunolabelling for urothelial differentiation-restricted antigens, such as the uroplakins and specialised tight junction proteins, can provide a surrogate marker of urinary barrier attainment – although it is important to be aware of inter-species differences (Chopra et al., 2008). A failure to present objective evidence of urothelial differentiation and/or barrier function can lead to discrepancies in interpreting bladder tissue-engineering studies.

In addition to its tight barrier function, a further notable feature of the urothelium is its regenerative capacity. When examined in situ, normal adult urothelium is a mitotically-quiescent epithelium that is able to undergo rapid proliferation in response to acute injury (Peyton et al., 2012, Varley et al., 2005). A controlled study of urothelial damage in rats conducted by Lavelle and colleagues demonstrated recovery of transcellular and paracellular components of the urinary barrier within 72 hours, with intermediate cells undergoing rapid maturation to form differentiated superficial cells (Lavelle et al., 2002). The excellent regenerative and differentiation capacity of urothelium is critical to maintaining the urine-proofing properties of the bladder and has positive implications for tissue-engineering strategies.

1.3 The clinical need for urinary bladder reconstruction

A diverse range of congenital and acquired chronic benign and malignant conditions can result in loss of urinary bladder function that then require some form of intervention to provide a urinary continence mechanism.

Incontinence is a socially-debilitating condition that affects some 400 million people worldwide (Irwin et al., 2011, Milsom et al., 2014). The condition is complex, but may arise due to direct or indirect interruption of the nerve supply to the bladder, for example due to congenital conditions of myelomeningocele/spina bifida or acquired conditions, such as multiple sclerosis and spinal cord injury. Other chronic benign bladder dysfunctions may be

consequential to vascular ischaemia caused by bladder outlet obstruction either during development or later in life, for example, acquired as the result of benign prostatic hyperplasia in older men. The resultant smooth muscle cell hypertrophy and increased connective tissue deposition causes thickening of the bladder wall. The effect on the biomechanics of the bladder wall is to render the bladder unstable, under high pressure, or lacking in capacity or compliance (German et al., 1994, Kruse et al., 1995, Watanabe et al., 1996). Consequent clinical problems can range from mild to severe chronic urinary incontinence, to irreversible kidney damage caused by the bladder being forced to work at abnormally high pressures and leading ultimately to renal failure requiring kidney transplantation.

Medical management aims to control the symptoms of bladder dysfunction with a range of drugs, including anti-muscarinics, alpha blockers, anti-diuretics and anti-cholinergics (reviewed in (Cameron, 2016)). Intra-detrusor injection of Botulinum Toxin A has become increasingly used, but requires repeat administrations (Fowler et al., 2012, Leitner et al., 2016, Utomo et al., 2014). Sacral neuromodulation/neurostimulation may be used in selected cases. However, a more permanent surgical augmentation of the bladder remains a clinical priority for those patients who develop a small-capacity, poorly compliant bladder, where intractable incontinence or pain destroys their quality of life, and/or where serious kidney damage is imminent (Biers et al., 2012, Cain and Rink, 2010).

Bladder cancer is one of the top ten most common cancer types in the world, with approximately 550,000 new cases annually (Richters et al., 2019). Patients requiring cystectomy (bladder removal) for high grade invasive bladder cancer represent a large group who can benefit from bladder reconstruction. Bladder removal may also be opted for by patients suffering unremitting bladder pain, which includes (mostly) females with idiopathic interstitial cystitis and also persistent abusers of ketamine, a drug which results in the destruction of the urothelium (Kidger et al., 2016). Common approaches for urinary diversion following cystectomy typically involve reconfiguring vascular pedicled bowel segments into orthotopic ileal neobladders, ileal conduit stomas or continent pouches (Stein and Skinner, 2006, Studer et al., 2004).

It is notable that the urinary bladder is not an organ that can be successfully transplanted, reflecting the vascular/neural dependency for maintaining bladder health and function. As

discussed in detail in the next section, the surgical augmentation of the bladder using bowel is considered the "gold standard" treatment for end-stage bladder disease, but significant clinical complications continue to drive research to find alternative options.

1.4 Urinary bladder reconstruction – the current "gold standard"

The most commonly performed surgical procedure for end-stage diseased bladders involves replacing or augmenting the bladder with a vascularised segment of the patient's own gastrointestinal (GI) tract, with the aim of creating a compliant, high capacity low-pressure neo-bladder. This procedure of **enterocystoplasty** involves isolating a segment of the GI tract on its vascular pedicle, detubularising it along the antimesenteric border to interrupt peristalsis, then reconfiguring and incorporating it into the bivalved bladder (augmentation cystoplasty) or as an orthotopic neobladder or conduit after cystectomy (Beier-Holgersen et al., 1994, Greenwell et al., 2001). The use of vascularised or pedicled host tissue grafts to augment the bladder in surgical reconstruction has a long history (Thomas, 1997, Budzyn et al., 2019), with latest developments seeing a shift towards bladder reconstruction using minimally-invasive laproscopic or robotic surgery (Nimeh and Elliott, 2018).

Despite success in augmenting bladder capacity, reducing intravesical pressures and improved continence, enterocystoplasty is associated with serious complications of bowel obstruction, mucus production, bladder calculi (stones), bladder perforation, metabolic acidosis and a possible increased long term risk of cancer (discussed {Khoury, 1992 #32;Thomas, 1997; Greenwell et al., 2001; Lima et al., 2015; Budzyn et al., 2019). As well as the morbidity associated with removing a section of bowel, a major issue is that gut epithelium is absorptive and mucus-producing in its normal function and, unlike urothelium, is not evolved structurally or functionally for long-term exposure to urine.

Attempts to avoid the complications of enterocystoplasty have included the use of free (non-vascularised) tissue grafts using fascia, split skin grafts, placenta, peritoneum, dura membrane and others in autologous/syngeneic settings in rats, rabbits, sheep, dogs and pigs (Neuhof, 1917, Draper et al., 1952, Fishman et al., 1987, Hutschenreiter et al., 1978, Kelami et al., 1970). Reported outcomes have been mixed, but include general problems of graft contraction and stone formation, alongside complications arising from the continued

normal functioning of the donor tissue (such as hair growth on skin grafts). The use of free tissue grafts is far inferior to enterocystoplasty, but is useful for highlighting the importance of vascularised tissue integration to prevent contraction and fibrosis of implants in the bladder wall.

2.0 Bladder reconstruction approaches using cells, biomaterials and tissue engineering

2.1 Objectives and approaches

The ideal tissue engineered urinary bladder should mimic the full range of functions fulfilled by the normal healthy bladder. During filling and voiding, the bladder undergoes dramatic changes in volume and is exposed to considerable mechanical forces (Korossis et al., 2009). Compliance of the bladder tissue is critical to accomplishing the low pressure storage of urine, as this protects the kidneys. Although an attractive long-term goal, the development of sensory self-voiding function is beyond current objectives and in the majority of bladder reconstruction strategies, it is anticipated that voluntary emptying will be aided by clean intermittent self-catherisation (CISC), either via the urethra or via an ileal conduit stoma or vesicostomy, such as described (Mitrofanoff, 1980).

The main approaches to bladder reconstruction can be categorised as biomaterials-based, cell-based or combined (tissue engineering) (FIGURE 2). The former, involving implantation of a biomaterial, is a passive approach that relies on the regenerative capacity of the host bladder for full integration, whereby the material becomes cellularised and is eventually resorbed and replaced. The alternative involves the active harvest (and possible in vitro expansion) of autologous cells from an appropriate host tissue, prior to surgical reimplantation, with or without a biomaterial 'scaffold' to provide structure. There are relative advantages and disadvantages to each approach, but the ultimate cost-effective solution would be an "off the shelf" biomaterial.

A major challenge with any approach is that where the underlying pathology of the host bladder is unresolved, neither passive functional biomaterial integration nor sourcing of healthy autologous cells for active tissue engineering approaches may be possible. In this context, it is noteworthy that most experimental tissue-engineering models use healthy

animals and problems can emerge when promising experimental research approaches are transferred to a clinical disease setting.

2.2 The cells

Although not the main purpose of this review, the sourcing of healthy autologous cells is critical to all cell-based or tissue engineering approaches. Adult autologous cells have several advantages over allogeneic cells in tissue engineering approaches, as the perfect genetic match excludes the need for immunosuppression. However, where the damage to the bladder reflects an underlying chronic disease process, it may not be possible to identify and harvest sufficient healthy autologous cells and in the case of urothelial cancer patients, the use of autologous cells from the urinary tract may not be safe. In such cases, alternative cell sources may be required for tissue engineered approaches to urinary tract reconstruction.

Subramaniam and colleagues examined urothelial cells harvested from paediatric patients with congenital neuropathic and non-neuropathic end-stage bladder diseases (Subramaniam et al., 2011). Whereas normal human urothelial cells are highly regenerative in vitro (Varley et al., 2005) and capable of differentiating to form a functional differentiated barrier urothelium (Cross et al., 2005), urothelial cells from end-stage diseased paediatric bladders showed reduced capacity for in vitro expansion and differentiation, indicating a need for alternative cell sources for engineered bladder reconstruction (Subramaniam et al., 2011). Given the desire to avoid life-long immunosuppression from the use of allogeneic cells, potential (but untested) solutions could include therapeutic modification of diseased cells during in vitro cell culture (eg using drugs or even CRISPR-Cas9 if a primary disease target or gene is known), use of a surrogate or transdifferentiated epithelium (Hustler et al., 2018), or potentially, the directed differentiation of functional urothelium from autologous induced pluripotent stem (iPS) cells (reviewed (Wezel et al., 2011)).

The challenges are even more stark when it comes to engineering complex multicellular tissues, such as the full bladder wall. Simplistic approaches taken to extract and grow in vitro a collation of the different cells present in the diseased fibrotic and inflamed bladder wall (Atala et al., 2006), without any cognisance of the conditions needed to reconstitute a functional, healthy tissue, seem in retrospect naive and doomed to failure (Joseph et al., 2014, Farhat, 2014).

2.3 Biomaterials

Biomaterials designed for direct or indirect contact with the human body are classified as medical devices under recognised International Standards (ISO 10993-1). The generic features of biomaterials are extensively reviewed (Williams, 2019) and more specifically, the "ideal" biomaterial for urinary bladder reconstruction has been described as biodegradable and with mechanical properties compatible with urinary bladder function, including compliance (sometimes mistakingly referred to as "elasticity") (Dahms et al., 1998, Elsawy and de Mel, 2017).

Most biomaterials are prepared from polymeric materials that are either natural or synthetic in derivation, with a further important class being acellular matrices (ACMs) which are rendered from natural tissues by decellularisation. To take each of these in turn:

Natural extracted soluble polymers include alginate (Rowley et al., 1999), hyaluronan (Arimura et al., 2005), chitosan (Drewa et al., 2008), fibrin (Hafez et al., 2005, Hafez et al., 2003) and collagen (Elbahnasy et al., 1998). Alone these materials typically show good biocompatibility but poor tensile properties, as demonstrated by Elbahnasy (Elbahnasy et al., 1998). Such materials have found use in various bladder reconstruction approaches, for example, as cell carriers (Hafez et al., 2005, Hafez et al., 2003), or in prevascularisation strategies (Hattori et al., 2006), whilst others have used them to produce hybrid materials in combination with reinforcing natural or synthetic fibres (Geutjes et al., 2006, Ajalloueian et al., 2014, Ajalloueian et al., 2013).

Synthetic polymers that are biocompatible and biodegrade to produce non-toxic residues have been investigated for soft tissue applications and include poly(ethylene glycol) (PEG) (Adelow et al.), poly(lactic-co-glycolic acid) (PLGA) and poly(ε-caprolactone) (PCL) {Baker, 2011 #66;Baker, 2009 #60}. The poly-α-esters are used commonly as the material has already been granted regulatory approvals for use in biomedical applications, for example as sutures (Pillai and Sharma, 2010). Synthetic polymers are particularly versatile as they are compatible with a wide range of processing technologies, including electrospinning (Baker et al., 2006), phase separation (Rowlands et al., 2007), gas foaming (Mooney et al., 1996), particulate leaching (Baker et al., 2011, McGlohorn et al., 2004), bioprinting (Serrano-Aroca et al., 2018) and chemical cross-linking (Park et al., 2002). This enables a huge variety of scaffolds to be created in knitted, felted or fabricated in other configurations with different

porosities, shapes and other features aimed at facilitating engraftment. Cell adherence is often used as an in vitro measure of biocompatibility, but there is a fundamental difference between 2D cell adherence to a substrate and the true 3D cell:cell and cell:matrix interactions that occur within a tissue (Baker et al., 2006). The production of nanofibrous scaffolds to mimic the 3D architectural form and scale of the tissue matrix has been attempted with synthetic polymers using three main fabrication techniques: electrospinning, molecular self-assembly and thermally-induced phase separation (Holzwarth and Ma, 2011, Asadian et al., 2020). In one study, electrospun scaffolds aimed at mimicking the scale and alignment of ECM fibres were predicted to create favourable conditions for cell:cell interactions for bladder smooth muscle engineering (Baker and Southgate, 2008); an approach tested by others in vivo and with partial success reported (Shakhssalim et al., 2017).

Typically, biomaterials are characterised by quantifiable physical properties such as pore size and interconnectivity, and chemical properties, including degradation rate and surface chemistry. Biomechanical properties such as stiffness are known to influence cell phenotype and tissue function, but any relationships are generally empirical. For example, PCL foams of 85-88% porosity and 35µm pore diameter produced by emulsion freeze-drying had a storage modulus that better approximated native bladder than equivalent foams constructed using PLGA. Cells grown on these different foams in vitro showed altered characteristics suggestive of different growth and contractile states (Baker et al., 2009). Nevertheless, it is poorly understood how to fine tune biomaterial properties to promote or enable particular biological responses, such as enhanced cellular and vascular engraftment or tissue-specific development, differentiation and function.

Discrepancies may also exist between in vitro and in vivo performances. For example, a porous, non-functionalised synthetic biomaterial generated by leaching sintered polystyrene spheres from a poly(ε-caprolactone) matrix showed limited propensity for cell infiltration in vitro, but was found to promote a strong stromal and angiogenic response in vivo (Baker et al., 2011), possibly due to architectural features such as “sweet spot” pore size (Ratner, 2016).

It is possible to functionalise polymer scaffolds, including nanofibrous scaffolds, by absorption, surface adsorption, chemical crosslinking or the indirect tethering of specific

growth and other bioactive factors, with the intent of enhancing biological behaviours from angiogenesis through to more complex behaviours (reviewed (Chen et al., 2010, Asadian et al., 2020)). The modification of surfaces by the covalent attachment of heparin offers one useful "biomimetic" approach as many growth factors are heparin-binding and tether naturally to heparin in their most natural, bioactive conformation (Rohman et al., 2009, Roelofs et al., 2018).

Natural acellular matrices (ACM) represent the insoluble matrix that remains after tissue decellularisation (Crapo et al., 2011, Keane et al., 2015). ACMs retain collagen, fibronectin, laminin, glycosaminoglycans and sequestered bioactive factors in original, tissue-specific architectures (reviewed (Davis et al., 2010, Gilbert et al., 2006, Marcal et al., 2012)). Such matrices are therefore predicted to provide a framework for homologous tissue regeneration following cell infiltration and remodelling by the recipient's own cells (Badylak, 2002). Nevertheless, it is important to appreciate that tissue structure has no blueprint, but is an emergent property of development. This raises questions about how effectively an implanted ACM will be remodelled into a functional tissue in vivo, particularly when implanted into a diseased tissue setting. Nevertheless, bladder ACM retains excellent compliance [FIGURE 3] and shows tissue- and immuno-compatibility, even when used in an allo- or cross-species setting. For example, in organ culture studies where human urinary tract tissue was combined with a porcine bladder-derived ACM (Bolland et al., 2007), infiltrating innate cells were polarised by bladder ACM to a tissue-integrative non-inflammatory M2 phenotype (Bullers et al., 2014). This presumably is a reflection of the high degree of conservation of the ECM between mammalian species.

ACMs have been reported to display batch and other variabilities (Ashley et al., 2010, Kropp et al., 2004) and/or carry risk of adverse reactions if there is inadequate removal of immunoreactive host material (Feil et al., 2006, Keane et al., 2012) or other potential xenopathological agents. Badylak's group from Pittsburgh proposed three minimum criteria for satisfactory decellularisation: 1) no visible nuclei upon histological evaluation using H&E and DNA stains; 2) the double stranded DNA (dsDNA) content to be <200 base pair fragment length; and 3) the amount of double-stranded DNA to be <50 ng per mg of dry weight of the material (Keane et al., 2015).

3. Review of bladder tissue engineering studies

Enterocystoplasty was first described in a canine model in 1888 and then in man a year later, but it was not until the mid-twentieth century that the technique became popular for the treatment of the contracted, tuberculous bladder. Stomach (gastrocystoplasty), small intestine (ileocystoplasty) and large intestine (colocystoplasty) have all been used as the vascularised reconstructing segment, with ileocystoplasty the most commonly performed procedure in the UK (Thomas, 1997).

3.1 Adaptations to enterocystoplasty

Given that the major side effects of enterocystoplasty are caused by the long-term exposure of the bowel mucosa to urine, a logical solution is to remove the bowel epithelium and leave the smooth muscle surface facing the lumen (seromuscular enterocystoplasty using demucosalised intestinal tissue). Performed experimentally in large animal (canine, porcine and bovine) surgical models, the latter approach has resulted in graft contraction, fibrosis, shrinkage, diverticulation and metaplasia, irrespective of which side of the bowel wall faces the lumen(Aktug et al., 2001, Clementson Kockum et al., 1999, Fraser et al., 2004, Hafez et al., 2005, Motley et al., 1990, Salle et al., 1990). These problems are thought to reflect severe inflammation secondary to urine exposure, infection of the graft and/or to ischemia or damage to the bowel segment during surgical dissection. There is some evidence that fibrosis may be avoided by retaining an intravesical silicone balloon for 2 weeks post-augmentation (Vilar et al., 2004), or more naturally, by finding strategies to cover the augmenting graft with urothelium (Aktug et al., 2001, Hafez et al., 2005, Turner et al., 2011).

Hafez and colleagues developed an aerosol transfer technique using suspensions of disaggregated bladder tissue cells in a fibrin glue (Hafez et al., 2003, Hafez et al., 2005). Autologous urothelial cells with or without smooth muscle cells, isolated at hemicystectomy, were sprayed onto deepithelialised colon and then incorporated into the remaining bladder. Studies assessed after 6 weeks (Hafez et al., 2005) and 6 months (Hidas et al., 2015) were described as resulting in a stratified, multi-layered uroplakin-positive urothelium atop of a bladder or colonic smooth muscle submucosa, respectively, and an absence of inflammation. An important limitation of this approach is the lack of “spare” healthy bladder tissue to provide adequate numbers cells of sufficient quality for the procedure.

Composite cystoplasty aims to get round the dearth of urothelium by expanding urothelial cells in vitro from small cyctoscopic biopsies, prior to reimplantation as cell sheets onto a pedicled de-epithelialised host smooth muscle segment used to augment the bladder. This approach exploits the regenerative nature of urothelium. First described in concept (Hutton et al., 1993), the approach was tested in a porcine in vivo model, first with sheets of undifferentiated urothelial cells (Fraser et al., 2004) and later with functional barrier-differentiated urothelial cell sheets (Turner et al., 2011). In each case, a PLGA (Vicryl™) mesh was used as a vehicle to transport the polarised urothelial cell sheets from cell culture to surgical site where it acted to protect the apical surface and facilitate integration of the basal surface with the host tissue. The latter study showed that implanting a differentiated (ie functional barrier) urothelial cell sheet prevented the inflammation seen when non-differentiated urothelial cell sheets had been used (Turner et al., 2011).

Neither the Hafez nor Fraser/Turner approaches described above have yet been reported to progress to clinical trials. Remaining challenges include the nature of diseased bladders requiring reconstruction, as features of the pathology, such as hypoxia (Radford et al., 2019) may impact on the tissue engineering potential of available autologous cells (Subramaniam et al., 2011) (and see section 2.2). However, a strategy that uses a preformed innervated and vascularised smooth muscle host tissue and only requires a single engineered tissue component, the urothelial urinary barrier, is less ambitious than attempting to engineer the entire bladder wall.

3.2 Bladder tissue engineering – the "Full Monty"

A number of investigators have described tissue engineering of the full bladder wall following subtotal (80%) cystectomy.

Atala and colleagues grew, then seeded autologous cells from the stroma and urothelium onto outer and inner surfaces of PGA mesh moulded into the shape of a bladder and coated with PLGA, (Oberpenning et al., 1999). The constructs were implanted in vivo onto a bladder base remaining after trigone-sparing cystectomy in dogs. Once coated with fibrin glue, the construct was wrapped with omentum and the animals monitored for up to 11 months. There were no reported complications and at three months, the polymer had degraded, leaving a vascularised, innervated tissue composed of organised smooth muscle bundles and a stratified urothelium, which was positive with antibodies against AUM. A

similar approach and two year findings were reported by Jayo and colleagues, who seeded a PLGA-based biodegradable synthetic polymer matrix with autologous urothelial and smooth muscle cells in a 80% sub-total cystectomy canine model. The constructs were reported to grow during skeletal maturation of the young animals (Jayo et al., 2008). The use of an omentum wrap appears to have been a critical step in both the Oberpenning and Jayo studies.

Atala and colleagues moved from their healthy canine model to test their approach in nine patients with severely neuropathic bladders (Atala et al., 2006). Collagen-only and collagen-PGA hybrid scaffolds were seeded with autologous stromal and urothelial cells and implanted. Three patients had a collagen-only implant, one had a collagen-only implant with an omental wrap and three patients had a collagen-PGA hybrid scaffold with an omental wrap. Two patients were lost to follow up and one patient with a collagen-only implant underwent conventional augmentation because of progressively rising intravesical pressures. The remaining patients were followed up annually for up to five years, although only four had investigations in the fifth year. The results showed minimal or modest increases in capacity and compliance of the bladders, with the best outcome in patients receiving cell-seeded collagen-coated PGA scaffolds that were wrapped in omentum as a vascular bed. The new bladder tissue was described as having a normal structure, with smooth muscle and stratified urothelium; however, the differentiation status of the urothelium was not reported. A follow up clinical trial was halted due to unacceptable adverse events (Joseph et al., 2014) and the approach heavily criticised as simplistic in an accompanying Editorial (Farhat, 2014).

3.3 ACM and other collagen-based matrices.

The two ACMs most studied for their potential application in bladder reconstruction are porcine small intestinal submucosa (SIS) and bladder acellular matrix (BAM), both of which were tested extensively in vitro and in preclinical surgical models before entering clinical trials (reviewed by (Kropp, 1998, Song et al., 2014, Pokrywczynska et al., 2015)).

SIS is prepared from porcine small intestine after mechanical delamination of the bowel wall to leave the collagen- and elastin-rich submucosal layer (Badylak et al., 1989). SIS has been variously employed as matrix alone, seeded with cells, or used in combination with synthetic or natural biomaterials. However, despite extensive and often promising in vitro and in vivo

studies (not reviewed here), results from clinical studies have reported unsatisfactory results in terms of bladder capacity, compliance, muscle regeneration and continence (Caione et al., 2012, Schaefer et al., 2013, Zhang and Liao, 2019).

BAM was first described in 1975 (Meezan et al., 1975). Despite initial promising results in small animals, transfer to a larger porcine model gave less favourable results, with contracture and failure of recellularization in the central areas of the implanted graft (Brown et al., 2002, Reddy et al., 2000). Further studies with cell-seeded BAM in a canine model (Yoo et al., 1998) provided proof of concept for a pilot human clinical study (Atala et al., 2006). However, the patients showed limited benefit, with deterioration in bladder capacity, compliance and leak point pressures.

Considering the major lessons to be learned from the SIS and BAM experiences, it is clear that graft size is one issue, and scaling from small to clinically-relevant implant sizes can overwhelm any regenerative properties inherent in a natural graft material. In rat models, for example, graft size is necessarily small (<0.5 cm^2) compared to a 4 x 4 cm^2 acellular dermal patch incorporated into a pig bladder (Akbal et al., 2006). A second issue, mentioned above, is that most surgical models involve healthy animals, whereas attempts to use disease models almost invariably fail (Zhang et al., 2006, Akbal et al., 2006), which leaves a wide gap when translating research findings to a clinical population. A final issue relates to the processing of natural materials as they transfer from research to more commercial clinical settings. For example, required terminal sterilisation procedures can have a profound negative effect on the properties of natural acellular matrices, leading to inhibition of cellular infiltration (Badylak, 2002, Bullers et al., 2014, Kimuli et al., 2004). Thus, despite early promise, ACMs have encountered problems of translation that have yet to be overcome.

3.4 Natural, synthetic and hybrid biomaterials

A number of studies have attempted to use collagen-based materials, recognising that as the major structural protein in the body, collagen is largely responsible for the strength and conformability of natural materials. Such studies have included the use of commercial collagen-based products such as INTEGRA™ (preparation of collagen type 1 and chondroitin-6-sulphate) (Parshotam Kumar et al., 2010) and OptiMaix (Leonhauser et al., 2017). Other groups have attempted to enhance the natural biocompatibility of collagen with growth

factors including VEGF, FGF2 or HBEGF (Roelofs et al., 2018), or insulin-like growth factor (Vardar et al., 2016). Whereas the Roelofs study showed no advantage of added growth factors, the Vardar study reported hypertrophy of the constructed urothelium, with a potential risk of urinary outlet obstruction. Overall, studies using collagen scaffolds, whether enhanced or not by cells or bioactive factors, have not yielded results that have led to clinical translation.

Synthetic materials should offer advantages over natural biomaterials in terms of reproducibility of composition, lower production costs and industrial scale up. Chemical inertness alone is no guarantee of successful bladder integration and implantation of synthetic materials (including plastics, polyvinyl sponge and polytetrafluoroethylene (Teflon™)) into the bladder have all met with failure to the point that the use of such materials is considered obsolete. As described in section 3.2, the use of synthetic materials in combination with cells from healthy animals has had some reported success in conjunction with the use of a vascularising omentum, but any potential was lost during translation to a diseased bladder patient population.

4. Conclusions and future trends

Reconstruction of the urinary bladder is carried out when conservative and medical therapies have failed to alleviate the debilitating symptoms of a small, non-compliant or diseased bladder. Surgical reconstruction with intestine (enterocystoplasty) remains the "gold standard" approach for providing patients with a continent, kidney-protecting reservoir, nonwithstanding the significant consequences of using an absorptive, mucus-producing bowel epithelium in place of a urothelial urinary barrier. For the present, improvements over enterocystoplasty via any biomaterials or tissue engineering route appears to have met an impasse. This failure to find a practical solution for bladder augmentation/reconstruction is disappointing given that it was back in 2006 when it was first announced that tissue engineered bladder replacement in children was a reality (Atala et al., 2006). The time since has seen the less-publicised failure of the Tengion®-sponsored clinical trial (Joseph et al., 2014) and reduced interest/funding for a clinical problem perceived by many to have been solved. So where do we go from here?

Substitution of the bowel epithelium by autologous urothelium remains an attractive strategy as it overcomes any need to engineer the complex vascularised smooth muscle component of the bladder wall. However, given the costs of personalised cell therapy, it is likely that a biomaterials approach would offer the more attractive cost-effective solution. Clinical practice is also likely to favour a cell-free reconstructive approach incorporating biomaterials alone. This will rely on developing suitable scaffold materials that both harness a tissue integration response and match the physical requirements of the bladder for compliance. These properties are currently most promisingly realised by bladder wall ACM, but understanding how integral matrix proteins such as collagens type I and III interact to develop the unique physical properties of the bladder matrix may facilitate the development of new nature-inspired materials. Finally, the route from laboratory to patient needs to be signposted to help promising novel products or strategies overcome the translational hurdle, particularly when transferring from a normal to disease state. One possibility is that modulation of the disease environment may provide a more conducive platform for rejuvenated cells to work with ACMs or nature-inspired scaffolds towards functional tissue development.

5 References

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Figures

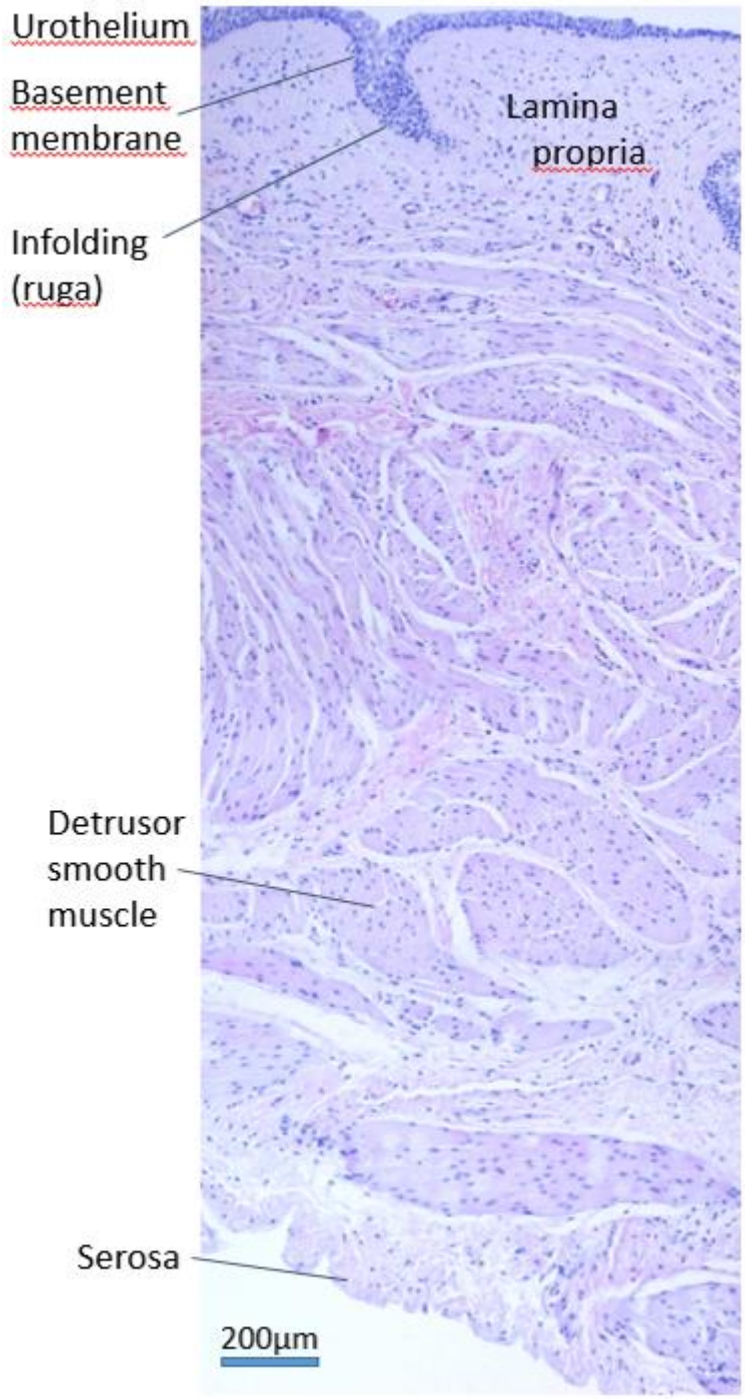

FIGURE 1. Transverse section through the porcine bladder stained with haematoxylin and eosin to show tissue features. Scale Bar 200µm.

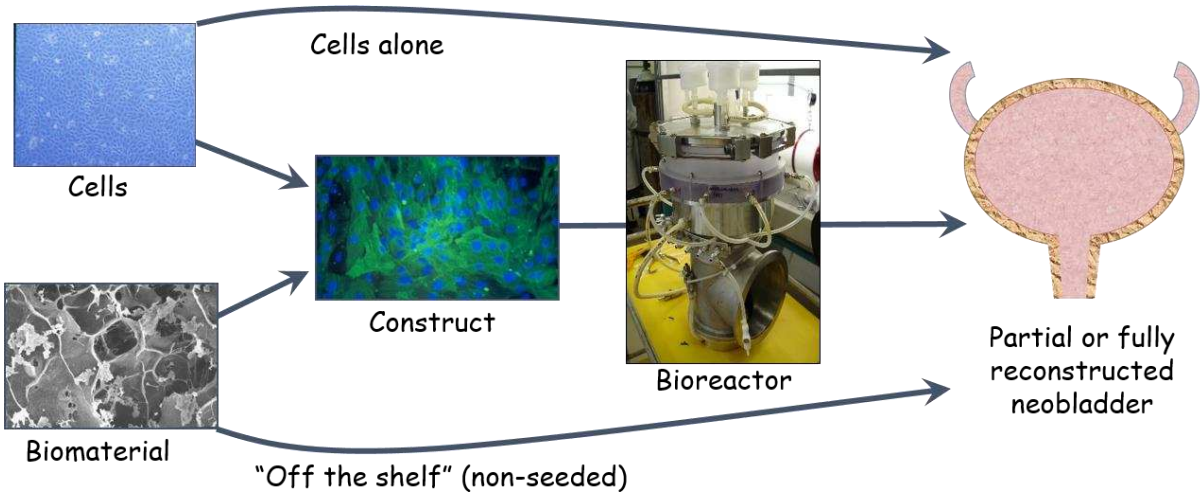

FIGURE 2. Strategies for bladder reconstruction can be categorised as biomaterials-based, cell-based or combined tissue engineering approach, where the generated tissue construct may be maintained and even functionally conditioned in a bioreactor prior to implantation in vivo. Notably, in some studies, the in vitro bioreactor may be substituted with an in vivo bioreactor, such as an omentum wrap.

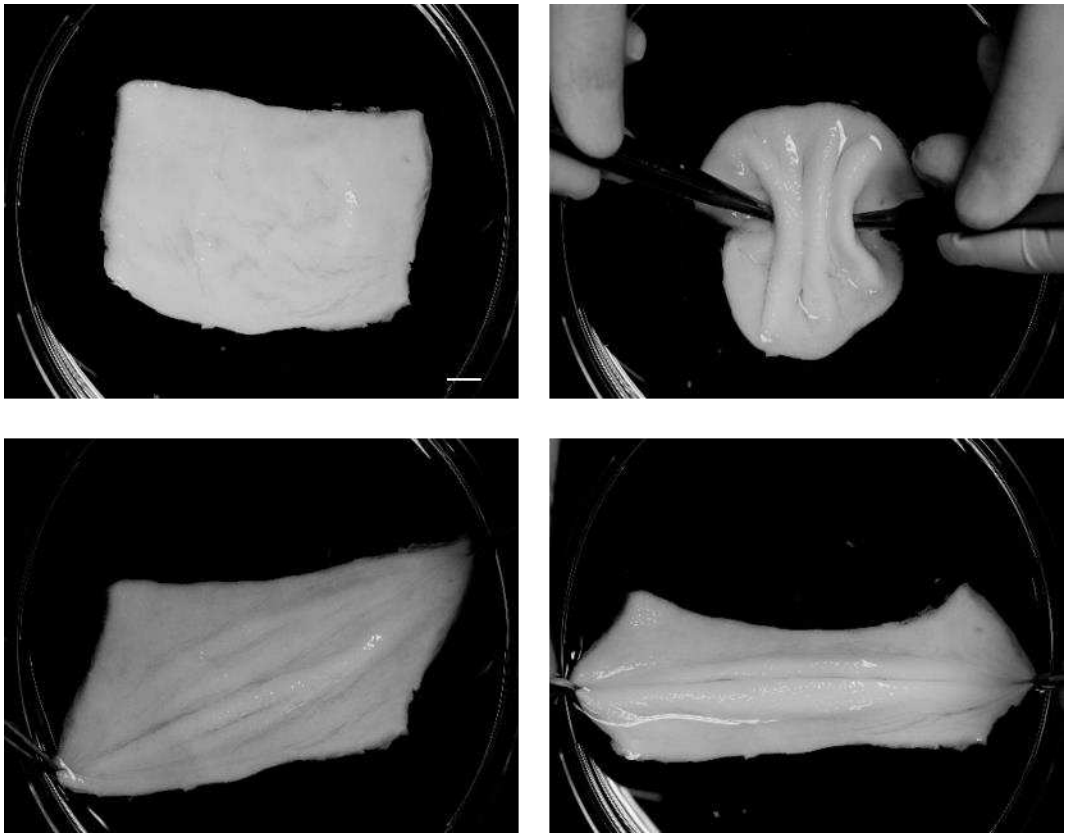

FIGURE 3. Macroscopic appearance of a natural matrix derived from porcine bladder by decellularisation, as described (Bolland et al., 2007). Decellularisation of the full thickness wall was achieved after distension and immersion of the intact bladder in a sequential series of sterile extraction buffers, including detergents and DNAse to lyse and remove cell components and render the tissue acellular. At the end of the procedure, the decellularised bladder is dissected open to present the biomaterial as a flattened sheet. The biomaterial retains many useful properties of the bladder wall including strength and compliance. Scale bar 1cm.