African Journal of Urology (2013) **19**, 51–57

Pan African Urological Surgeons' Association

African Journal of Urology

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Review

Fundamentals of bladder tissue engineering

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Received 15 October 2012; received in revised form 10 December 2012; accepted 7 January 2013

KEYWORDS
Scaffold;
Stem cells;
Bladder tissue engineering;
Decellularization;
Bladder acellular matrix

Abstract
A wide range of injuries could affect the bladder and lead to eventual loss of its integrity, with the need for replacement or repair. Augmentation ileocystoplasty is considered till now the gold standard option for bladder replacement, despite its associated complications. Bladder tissue engineering appears as an appealing alternative through development of biological substitutes, which could restore structural and functional aspects of damaged tissues and organs.
Tissue engineering relies upon three essential pillars; the scaffold, the cells seeded on scaffolds and lastly the environmental conditions, including growth factors, cytokines and extracellular matrix (ECM) which promote angiogenesis and neurogenesis of the regenerated organs. The choice of the scaffold and the type of cells is a crucial and fundamental step in regenerative medicine. In this review article, we demonstrated these three crucial factors of bladder tissue engineering, with the pros and cons of each scaffold type and cell type used.

Introduction

The bladder could be damaged or lost via wide range of injuries, necessitating subsequent replacement or repair. Children with high-pressure low compliant bladders as a result of congenital anomalies such as bladder exstrophy and myelomeningocele always succumb to augmentation cystoplasty when medical treatment fails [1].

Gastrointestinal segments are always used as donor tissues for augmentation cystoplasty. However, several deleterious complications can occur, such as electrolytes imbalance, urolithiasis, mucus production, and malignancy [2].

Due to the aforementioned problems associated with gastrointestinal segments application, investigators and researchers have attempted to use alternative methods, materials, and tissues for bladder tissue engineering. In 1917, Neuhof was the first one to report application of a free tissue graft for bladder replacement. He incorporated fascia to augment bladders in dogs [3]. This was followed afterwards by a series of experiments and clinical

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http://dx.doi.org/10.1016/j.afju.2013.01.006

trials using several other materials as free grafts, including skin, bladder submucosa, omentum, dura, peritoneum, placenta, seromuscular grafts, and small intestinal submucosa [4–9]. Synthetic materials such as polyvinyl sponge, tetrafluoroethylene, gelatin sponge, collagen matrices, vicryl matrices, resin-sprayed paper, and silicone were also tested experimentally and clinically [10–12].

The previously mentioned trials have usually failed due to mechanical, structural, functional, or biocompatibility issues. Bladder tissue cannot be easily replaced due to its elasticity and urothelial permeability. Subsequently, bowel tissue remained as the gold standard option for more than a century after it was proposed, despite associated complications. Anastomoses between sets of urological tissues are considered the best functional alternative, but paucity of autologous urological tissues for reconstruction remains an obstacle [13]. Therefore researchers were urged to seek alternative solutions as tissue engineering due to lack of an entirely satisfactory clinical option [1].

Tissue engineering

Bladder tissue engineering appears as an appealing alternative through development of biological substitutes, which could restore structural and functional aspects of damaged tissues and organs. It employs aspects of cell biology, transplantation, and biomedical engineering.

Components of tissue engineering

Tissue engineering relies upon three essential pillars in the organ regeneration process:

(1) The biomaterial or the scaffold.
(2) The cells seeded on the surface of the biomaterial.
(3) The environmental conditions, including active growth factors [vascular endothelial growth factor (VEGF), transforming growth factor β1 (TGFβ1), epidermal growth factor (EGF), transforming growth factor α (TGFα), etc.], cytokines and extracellular matrix (ECM) which promote angiogenesis and neurogenesis of the regenerated organs.

Therefore the choice of biomaterial or scaffold and the type of cells is a crucial and fundamental step in regenerative medicine.

Biomaterials or scaffolds

Why do we use biomaterials in tissue engineering?

The biomaterial design is fundamental in the development of engineered genitourinary tissues. The type of the biomaterial plays a critical role in controlling the structure and function of the engineered tissue in a preplanned manner by interacting with transplanted cells or host cells.

Biomaterials facilitate both the localization and delivery of cells and/or bioactive factors to desired non-functional sites of the body. They can also act as a three-dimensional (3D) cytoskeleton for the development of neo-tissues of appropriate structure, and lastly they can guide the formation of functional new tissues [14]. In some instances, direct injection of cell suspensions without biomaterial matrices has been used [15,16], but it showed technical difficulty to control the localization of transplanted cells.

Appropriate regulation of cell behavior (e.g. adhesion, proliferation, migration, differentiation) by the biomaterials is crucial to promote the genesis of functional new tissue. Cell behavior is regulated by multiple interactions with the microenvironment, including interactions with cell-adhesion ligands [17] and with soluble growth factors [18]. These can be provided by the biomaterial itself or integrated into the biomaterial to control cell behavior [19].

Bladder scaffolds used must be able to support the adhesion and proliferation of specific cell types, including urothelial cells (on the luminal side) and smooth muscle cells which surrounds the urothelium, and it must be able to direct proper tissue development and differentiation in order to form a functional adaptive compliant bladder.

To withstand forces exerted by the surrounding tissues, the biomaterials must provide temporary mechanical support. In the case of bladder replacement, the biomaterial must be able to withstand forces resulting from bladder filling and evacuation. In addition, the biomaterial must withstand the forces exerted on it by the pelvic musculature during the patient's daily activities. Mechanical support of the biomaterials should be sustained till the engineered bladder has sufficient mechanical integrity to acquire its complete functionality [20].

Finally, the biomaterial must have certain properties to be processed into different conformations. It must be molded into a tubular shape in cases of urethral replacement, or into a hollow spherical configuration in cases of bladder replacement.

Characteristics of ideal scaffold

The ideal scaffold should be non-toxic, have the same mechanical properties as the tissue of interest, and integrate biochemical, spatial and topographical cues in a hierarchical manner to replicate the properties of native tissue (adhesive cues, mass transport, surface texture and composition)

Types of biomaterials

Generally speaking, three major types of biomaterials have been used for engineering of genitourinary tissues [21]; these are:

(a) Naturally derived materials, such as collagen and alginate.
(b) Acellular tissue matrices, such as bladder submucosa (BSM) and small-intestinal submucosa (SIS).
(c) Synthetic polymers, such as polyglycolic acid (PGA), polylactic acid (PLA) and polylactic-co-glycolic acid (PLGA).

These types of biomaterials have been tested for biocompatibility issues with primary human urothelial and bladder muscle cells [22]. Naturally derived materials and acellular tissue matrices have the potential advantage of biologic recognition, while synthetic polymers can be produced enormously and quickly with controlled properties of strength, degradation and microstructure.

Naturally derived scaffolds. Collagen is purified from both animal and human tissues, and it is considered the most abundant structural protein in the body. It shows minimal inflammatory and antigenic responses [23] and it has been approved by the Food and Drug Administration (FDA) for many types of medical applications [24]. To be less vulnerable to the enzymatic degradation, intermolecular cross-linking is of proven efficacy. Cross-linking can be done

by either physical (e.g. ultraviolet radiation) or chemical (e.g. glutaraldehyde) techniques [25]. On the other hand, alginate is utilized as an injectable cell delivery vehicle [26] and a cell immobilization matrix due to its gelling properties.

A new innovative technique has been adopted recently using alginate and collagen. They are used as 'bio-inks' in a newly developed bio printing technique based on inkjet technology [27,28]. These constructed scaffold materials can be 'printed' into desired scaffold configurations using a modified inkjet printer. This new modality can be further upgraded so that a 3D construct containing a certain precise arrangement of cells, growth factors and ECM material can be printed [29]. Furthermore, these constructs can be implanted into a host serving as the backbone for a new tissue or organ.

Acellular tissue matrices. Acellular tissue matrices are collagen-enriched matrices prepared by removing antigenic cellular contents, a process known as decellularization, using different mechanical and chemical processes [30,31]. The resultant ECM retains most of the functional and structural proteins, glycosaminoglycans (GAGs), glycoproteins, and bioactive factors [32]. The rationale for using such a scaffold in tissue engineering is that the resultant scaffold would possess the same composition, mechanical properties and complexity as the native tissue and integrate all these cues in a hierarchical manner, bypassing the need to engineer them into an artificial scaffold. Moreover, acellular matrices from allogeneic, cadaveric and even xenogeneic sources could be used as most antigenic proteins are removed by the decellularization [33]. To summarize, the resulting ECM shows all the criteria of the optimum scaffold used in tissue engineering [34].

As acellular matrices degrade over time after process of implantation, they are remodeled by native ECM proteins or by ECM synthesized by transplanted cells. In previous trials, acellular tissue matrices have been shown to support cell ingrowth and regeneration of several genitourinary tissues, including urethra and bladder. They did not exhibit any immunogenic rejection [7,35].

Acellular matrices show a wider variation in composition and physical moduli (such as tensile strength, elasticity and breaking strength) as compared to synthetic scaffolds because it is derived from animal sources. Many components of native intact bladder are found in bladder acellular matrix (BAM), including laminin, fibronectin, entactin, decorin, and collagens I, III, IV, V and VI [36].

BAM is considered as one of the most representative decellularized tissues. It has been extensively utilized in bladder tissue engineering in various animal models. It induced the ingrowth of several cell types, including urothelial cells, smooth muscle cells, endothelial cells, and nerve cells. However, there were still distinguishable poorly organized smooth muscle cells in the scaffold center in bladders augmented with BAMs alone. Inadequate vascularization occurring in these augmented bladder tissues predisposed to bladder fibrosis and eventually affected the long-term bladder function [37].

Synthetic polymers. These synthetic polyesters of naturally occurring α-hydroxy acids are widely used in regenerative medicine. End-products of these polymers are non-toxic, and are eliminated from the body in the form of carbon dioxide and water [38]. Owing to the thermoplasticity of these polymers, they can be easily reconstructed into a 3D scaffold of specified microstructure, shape and dimensions [39,40].

Graft failure associated with use of synthetic polymers may manifest by recurrent urinary tract infections, calculi formation, contracture of grafts and graft rejection [41,42]. Moreover, although these polymers can be processed into complex three-dimensional structures, they do not contain cues promoting cell adhesion, proliferation and differentiation and do not display the same elasticity as detrusor muscle. Another major side effect of these synthetic polymers is lack of biologic recognition, predisposing them to be attacked by the body immune system [19].

Composite scaffolds consisting of both natural and synthetic materials have been developed and may be useful in genitourinary tissue engineering. In particular, these scaffolds may be useful for engineering organs that are composed of layers of cells, such as the bladder (urothelial layer surrounded by smooth muscle cells) [43].

Cells

The ability to use donor tissue efficiently and to provide the right conditions for long-term survival, differentiation and growth are fundamental steps for successful cell transplantation strategies in bladder tissue engineering. Various cell sources have been used for bladder regeneration. Bladder neck and trigone areas have a higher concentration of urothelial progenitor cells [44], being localized in the basal region [45]. Amniotic fluid and bone marrow-derived stem cells can also be used in an autologous manner and have the potential to differentiate into bladder muscle [46] and urothelium [47]. Embryonic stem (ES) cells also have the potential to differentiate into bladder tissue [48].

Types of cells used in tissue engineering

Differentiated native cells from the organ of interest (examples are urothelial and smooth muscle cells)

One of the technical limitations for applying cell-based regenerative medicine was the inherent difficulty of growing certain human cell types in large quantities. Native targeted progenitor cells are found in every organ of the body, and this led to improved culture techniques overcoming this technical problem. These cells are tissue-specific unipotent cells, being derived from most organs [49].

In the past, urothelial cells were cultured in vitro, but with only limited success. Over the last two decades, several protocols and trials have been tested with improved success rates in urothelial growth and expansion [50,51]. Using modified cell culture techniques; it now feasible to culture an urothelial strain from a single specimen that initially covers a surface area of 1 cm^2 to one covering a surface area of 4202 m^2 within 8 weeks [51].

Native targeted progenitor cells are already programmed to become the cell type needed when implanted in the tissue of interest, requiring no in vitro differentiation. They can also be obtained from the specific organ to be regenerated, expanded and used in the same patient without rejection, in an autologous manner, obviating the issues of biological recognition [50–52].

Major concerns arose when using native progenitor cells. If cells were expanded from a diseased organ, there may be no longer

enough normal cells in that organ to be obtained. Lin et al. showed that cultured neuropathic bladder smooth muscle cells possessed and maintained different characteristics than normal smooth muscle cells in vitro [53]. On the contrary, Lai et al. were able to demonstrate that matrices seeded with in vitro cultured neuropathic smooth muscle cells, and then implanted in vivo, showed the same properties as the constructs engineered with normal cells [54].

Genetically normal progenitor cells are present even in diseased tissue. They are considered as reservoirs for new cell formation, being programmed to give rise to normal tissue, regardless of whether they reside in a normal or diseased environment.

Stem cells

Due to the aforementioned obstacles associated with utilizing urothelial and smooth muscle cells, stem cells are considered to be ideal candidates for tissue engineering.

Types of stem cells used in tissue engineering
Embryonic stem (ES) cells

Pluripotent human stem cells are an ideal source of cells, as they can differentiate into any replacement tissue in the body. One example is ES cells. These cells have two important remarkable properties: the ability to proliferate in an undifferentiated state, but still pluripotent (self-renewal) and the ability to differentiate into a wide range of specialized cell types [55]. Drawbacks of using ES cells include their propensity to form teratomas when implanted in vivo due to their pluripotent state, and the cells are not autologous, thus limiting their clinical application due to problems with biological recognition and rejection [49].

Adult stem cells

Adult stem cells are the best-understood cell type in stem cell biology [56]. They could be retrieved from different adult tissues, including the brain, heart, lungs, kidney, and spleen [57]. The most well characterized source for adult stem cells is adult bone marrow. Adult bone marrow contains a heterogeneous group of cells, including hematopoietic stem cells, macrophages, erythrocytes, fibroblasts, adipocytes, and endothelial cells. In addition to these cell types, non-hematopoietic stem cells exist possessing a multilineage potential. These stem cells are commonly called marrow stromal stem cells or mesenchymal stem cells, and more commonly now, mesenchymal stromal cells (MSCs). Originally, MSCs are primitive cells which derive from the mesoderm, being able to differentiate into connective tissues, skeletal muscle cells, and cells of the vascular system [57]. Classifying MSCs as non-hematopoietic, multipotential stem cells make them capable of differentiating into both mesenchymal and non-mesenchymal cell lineages [57].

MSCs home to and engraft to injured tissues. They synergistically down-regulate pro-inflammatory cytokines and upregulate anti-inflammatory factors thus modulating the inflammatory response. Moreover, MSCs demonstrate immunosuppressive properties via suppressing T-cells, natural killer (NK) cell functions, and modulating dentritic cell activities [57].

Multipotent bone marrow-derived MSCs are attractive candidates for bladder tissue engineering as they can directly differentiate into smooth muscle cells. They could also act through a paracrine effect, as they are known to secrete a variety of pro-angiogenic, pro-regenerative and mitogenic cytokines favoring tissue regeneration.

It has been shown that human bone marrow MSCs can also differentiate into smooth muscle cells (SMCs) with the use of fetal bovine serum (FBS) and TGFβ1 [58]. The regenerative potential of bone marrow MSCs to replace tissue through their differentiation into smooth muscle cells (SMCs) has been studied by several groups. Shukla et al. have cultured porcine MSCs and differentiated them in vitro into mature SMCs. They showed that the labeled MSCs survived 2 weeks after implantation [46].

Amniotic-fluid and placental-derived stem cells (AFPS)

Multipotent amniotic-fluid and placental-derived stem (AFPS) cells are capable of extensive self-renewal. They represent 1% of the cells found in the amniotic fluid and placenta. The undifferentiated stem cells expand extensively without a feeder cell layer and double every 36 h. However, AFPS cells do not form tumors in vivo [49]. AFPS cell lines can be induced to differentiate into cells from all three germ cell layers, including cells of adipogenic, osteogenic, myogenic, endothelial, neural-like and hepatic lineages [59].

These cells could be obtained either from amniocentesis or chorionic villous sampling in the developing fetus, or from the placenta at the time of birth. De Coppi et al. were able to show that muscle-differentiated AFPS cells prevented compensatory bladder hypertrophy in a cryo-injured rodent bladder model [60]. AFPS cells represent a new class of stem cells with properties somewhere between those of ES and adult stem cell types. They are more active than adult stem cells, but less so than ES cells.

Adipose-tissue derived stem cells (ADSCs)

Adipose tissue is derived from mesoderm germ cell layer. It contains a supportive connective tissue stroma of pluripotent progenitor cells referred to as ADSCs. These stem cells have the ability to differentiate into the three germ cell layers including, myogenic, adipogenic, osteogenic, chrondrogenic, and neurogenic lineages in vitro. A major advantage in using ADSCs is that they are enormously abundant and easily accessible [61], unlike other cell types used in tissue engineering. They can be easily obtained via liposuction procedures using local anesthesia, and with minimal morbidity [62]. An aspirate of adipose tissue contains approximately 3% of ADSCs. In comparison, the frequency of similar cells in bone marrow aspirates is three times less [63].

The multipotentiality of ASCs, their ease of obtainment, and ability to differentiate into functional and contractile smooth muscle make them an attractive source for bladder tissue engineering. In several animal studies that are recently published or in press, ADSCs have demonstrated efficacy in the treatment of various types of dysfunctional bladder and urethra [64,65].

Growth factors

Growth factors initiate multiple effects involved in various aspects of cell functioning, from reproduction and differentiation to apoptosis. They are sometimes called mitogens because they stimulate mitotic division of the cell. Some of them are universal, whereas others are specific for certain cell types.

Growth factors can accelerate the reproduction of MSCs and induce proliferation of resting clonogenic cells, which promotes either conservation of their undifferentiated phenotype or transition into the state of commitment. The same effects stimulate some

variants of their differentiation. Epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), and platelet-derived growth factor (PDGF) belong to the factors studied enormously. Proliferation and differentiation of MSCs are also influenced by some other growth factors whose effects, however, are less significant [66].

Clinical trials in bladder tissue engineering

In the last decade several attempts to engineer urological tissue have been reported. Furthermore, trials have been undertaken to reconstruct even higher complex tissues, such as renal structures, corpora cavernosa, and vaginal tissue. However, only few of these approaches have advanced beyond animal experiments to human clinical studies. The most noteworthy study to date on the clinical applications of bladder substitutes is that of Atala et al. [1]. Patient-specific bladder tissue substitutes were created from autologous bladder tissue of seven patients with myelomeningocele who required bladder augmentation. Patients first underwent open bladder biopsies 6 weeks prior to implantation for retrieval of smooth muscle and urothelial cells. The two components were expanded in vitro, seeded onto acellular collagen-based scaffolds, and then successfully implanted into the respective patients. At a mean follow of 46 months, improvement in bladder capacity was evident. Histologic analysis at 5-year follow up displayed tri-layered architecture comprised of urothelium, submucosa, and muscle. It is important to note that these patients had end-stage bladder disease from myelodysplasia and they were dependent on intermittent catheterization to achieve complete bladder emptying prior to surgery. Although evidence of possible neural regeneration was seen in these tissue constructs, the potential for postoperative volitional voiding remains to be seen.

Conclusion

Bladder tissue engineering requires manipulation of different kinds of cells, and various scaffold types, to share in the final outcome of the engineered bladders, as regards the functionality and durability. Bone marrow MSCs, Skeletal MSCs, ADSCs and AFPSCs have been tested in preclinical trials for bladder augmentation and detrusor regeneration with various degrees of efficacy. Retrieval of stem cells, especially bone marrow MSCs appears to be feasible, bypassing the antigenic aspects and relatively inexpensive. Adipose tissue is another source of abundant and easily obtained stem cells, however incorporation into more preclinical trials is paramount before justifying their functionality, efficacy and durability. The use of bladder acellular matrices is another alternative viable option when considering scaffold use. However, prospective randomized trials will dictate both the short-term and long-term outcomes of these cells and scaffolds.

Despite these advances, challenges facing urology and other medical disciplines are numerous. In regard to ES cells, ethical and tumorigenicity concerns are paramount. As regard to adult stem cells, their exact mechanism of action is still hypothetical; is it true transdifferentiation leading to replenishment of degenerated tissue? Or, do these stem cells secrete certain growth factors that help the host tissue to regenerate? Answers to these questions will direct future trials and will eventually widen the scope for further use of tissue engineering in regenerative medicine as a whole.

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Akademii nauk. Seriia biologicheskaia/Rossiiskaia akademiia nauk 2008;6:645–62.

Glossary of abbreviations

VEGF: vascular endothelial growth factor

TGFβ1: transforming growth factor β1

EGF: epidermal growth factor

TGFα: transforming growth factor α

ECM: extracellular matrix

BSM: bladder submucosa

SIS: small intestinal submucosa

PGA: polyglycolic acid

PLA: polylactic acid

PLGA: polylactic-co-glycolic acid

FDA: food and drug administration

3D: three dimensional

GAGs: glycosaminoglycans

BAM: bladder acellular matrix

ES cells: embryonic stem cells

MSCs: mesenchymal stromal cells

NK cells: natural killer cells

SMCs: smooth muscle cells

FBS: fetal bovine serum

AFPS: amniotic fluid and placental derived stem cells

ADSCs: adipose-tissue derived stem cells

bFGF: basic fibroblast growth factor

PDGF: platelet-derived growth factor