REVIEW PAPER

FUNCTIONAL UROLOGY

Tissue engineering of urinary bladder – current state of art and future perspectives

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Introduction. Tissue engineering and biomaterials science currently offer the technology needed to replace the urinary tract wall. This review addresses current achievements and barriers for the regeneration of the urinary blad- der based on tissue engineering methods.
Materials and methods. Medline was search for urinary bladder tissue engineering regenerative medicine and stem cells.
Results. Numerous studies to develop a substitute for the native urinary bladder wall us- ing the tissue engineering approach are ongoing. Stem cells combined with biomaterials open new treatment methods, including even de novo urinary bladder construction. However, there are still many issues before advances in tissue engineering can be introduced for clinical application.
Conclusions. Before tissue engineering techniques could be recognize as effective and safe for patients, more research stud- ies performed on large animal models and with long follow–up are needed to carry on in the future.

Key Words: stem cells ‹› bladder regeneration ‹› tissue engineering

INTRODUCTION

Approximately 400 million people worldwide suffer from urinary bladder cancer (BCa). Approximately 25 to 30% of BCa patients suffer from muscle invasive disease and about 75 to 80% of them need radical cystectomy [1]. In Poland, BCa is the fourth most common cancer in men and the eighth most common cancer in women [2]. Approximately 3,000 of new cases of BCa are expected in Poland in 2013. Cystectomy is most commonly performed to treat invasive cancer of the bladder, however, BCa is not the only indication for this procedure. Intestinal cystitis, congenital abnormalities, trauma, and infection can lead to urinary tract injuries that require reconstructive surgery to be performed. When radical cystectomy is required as treatment, a replacement material is necessitated [3]. Commonly used in clinical practice is an approach that utilizes the intestine for urinary bladder reconstruction. The overall rate of complications after cystectomy is estimated at 25 to 35%, including major complications that occur at a rate of 5%. The rate of radical cystectomy related deaths is 1 to 3%. The deleterious effects associated with the use of bowel include obstructive nephropathy, formation of bladder stones, organ prolapse, infection, urinary incontinence, erectile dysfunction, carcinogenicity, electrolyte abnormalities, intestinal obstruction, and perforation [4].
The need to find the new method for urinary bladder reconstruction is eminent for modern urology. Regenerative medicine holds great promise for achieving this challenge. The regenerative medicine approach, based on tissue engineering methods, assumes bladder reconstruction through bladder regeneration [5]. This idea involves primary *in vitro* seeding of stem cells on a biodegradable scaffold in order to achieve bladder wall regeneration.

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Implementation of the *in vitro* constructed grafts for the treatment of patients is with the hope of reducing the number of future post–operative complications and *hospitalizations and, thus, improving the quality of life after cystectomy and reducing the costs of treatment.*

Regenerative Medicine & Tissue Engineering

Regenerative medicine is a broad term that includes the replacement, repair, and regeneration of tissues and organs. Replacement means growing *in vitro* tissues and organs for later implantation to the recipient. Repair refers to restoring proper tissue function by intervening at the molecular level. Finally, regeneration means growing a new tissue *in vivo*. Urologic tissue engineering is focused on replacing damaged parts of the urinary tract in order to restore its function [6].
Regenerative medicine has been recognized as a priority direction of medical science development in the USA and Germany. The Polish Regenerative Medicine Society was set up on the 27th of May, 2011 by Professor Mariusz Ratajczak. Regenerative medicine is a promising field of medicine because it is an answer to many urgent medical needs. It is already evident that this emerging field will be crucial for the development of medicine and biotechnology in our country. Due to the advanced level of research and innovative infrastructure required for regenerative medicine therapies, there is a need for highly qualified executives with prior expertise in stem cell and transplantation biology as well as extensive experience in related areas of translational research [7]. Despite its great potential for growth, regenerative medicine and cell–based therapies face many challenges in the areas of funding and implementation caused by the highly innovative research phase.
The regeneration of a previously removed urinary bladder is a very promising vision that may soon become viable due to advances in regenerative medicine. Emerging therapies addressed to patients with urinary bladder may revolutionize urology. Modern urology does not offer effective methods to restore urinary bladder function after cystectomy. Such patients struggle with side effects that need expensive hospital treatment [8]. The average age of patients with bladder cancer is 60 years [9]. In today's aging society these people are an important group of the working–age population. Regenerative medicine therapies offer treatment for those who are unfit for employment due to health disorders after cystectomy. The restoration of urinary bladder function is crucial for proper function of the urinary system, which makes an important contribution to homeostasis. The important benefit from investing in regenerative medicine research is providing treatment for health problems of the ageing population, which affect macroeconomic performance through both the labor and capital markets.
The number of new cases of bladder cancer in Poland has increased by 50% over the last two decades [10]. Atala et al. reported in 2006 that *in vitro* constructed neo bladders were successfully implanted in 20 patients. However, since then no new tissue engineering–based therapy has been introduced into clinical practice. Nevertheless the major advantage of the study presented by Atala et al. is that it brought evidence that replacement of human urinary bladder is possible to perform [11]. Nowadays, we have more data gained from tissue engineering and biomaterials research to improve technology of urinary bladder regeneration in order to develop effective treatment that is suitable to be used in broad clinical practice.

Biomaterials

Tissue engineering applies the performance of modern biomaterials to create cell matrices that support stem cells survival, proliferation, and further tissue regeneration [12].
Biomaterials research for bladder reconstruction began in the 50s when acrylic mold were demonstrated for urinary bladder wall replacement [13]. Together with progress in the field of biomaterials, the following were evaluated for applicability in urinary bladder reconstruction: polyvinyl sponge, polyethylene mold, Teflon mesh, silastic patches, gelatin sponge, collagen, and vicryl [14]. However, the results of the studies performed on animal models were not sufficiently satisfactory to plan clinical trials. In recent years many biomaterials were proposed for urinary bladder reconstruction. Although the published results showed that reconstructed urinary bladders functioned well, none of these experimental methods were developed further. Only SIS (Small Intestinal Submucosa) was employed to urinary bladder reconstruction in clinical practice. However, SIS is no longer considered to be suitable material for urological application. Conducted studies showed that SIS is cytotoxic for urothelial cells and in turn inhibit urothelial layer regeneration [6].
The current point of view on biomaterials in regenerative medicine has evolved to the molecular level of creating bioactive materials that impact certain cellular events. Biomaterials should mimic the ability of the extracellular matrix (ECM) to regulate cell functions such as cell division, differentiation, and apoptosis. It is particularly important when trying to regenerate urinary bladder. An effective process of regeneration should follow the steps of organogenesis. During organogenesis the urothelial cells regulate smooth muscle layer formation due

to paracrine and direct cell–to–cell interaction conducted via the ECM. The ECM also regulates the direction of migration and cell differentiation due to proteoglycan and growth factor content. The graft environment should make the restoration of these interactions possible. This requirement can only be met after using an adequate scaffold for the *in vitro* constructed graft. Nanotechnology brings the opportunity to achieve these goals through rendering material structure at the nano scale. In recent years review articles have been published whose authors call for the use of electrospun fibers for application in reconstructive urology. Bioengineered grafts composed of electrospun biomaterials and stem cells were proven to induce urinary tract regeneration after implantation. Most of these studies used rats as an animal model for partial or radical cystectomy, concerning regeneration of urinary bladder smooth muscle layer using scaffolds made from elctrospun poly(1,8–octanediol–co–citrate) nanofibers, fibrogen nanofibers, or collagen nanofibers were demonstrated to support the regeneration of urinary bladder and reduce scar formation. The nanofibers were documented to improve graft angiogenesis [15, 16]. Angiogenesis is a key factor needed to create a suitable environment for urinary bladder regeneration. Most of the studies that concerned urinary bladder regeneration using nanofibers were conducted based on small animal model – mainly rat [17, 18, 19]. It is an important reason why, despite the great variety of tissue engineering methods proposed for urinary bladder augmentation, none of them entered into clinical use. There is still not enough research evidence to propose a therapy for clinical use. More research data should be collected from studies conducted on large animal model whose urinary system histology structure and physiology is similar to the human one. The urinary bladder of rat is not a suitable model for evaluation of urinary bladder histological structure and function. The results obtained after reconstruction of rat's urinary bladder do not reflect the function of human urinary bladder after a similar procedure. The side effects that may have occurred in patients are also hard to predict. It is high time to translate evidence into clinical practice and begin to use it more frequently on large animal models for experimental research in the field of reconstructive urology to obtain more reliable data.

Stem Cell

Summarizing results of studies for bladder wall regeneration from the last decade, it becomes clear that stem cells are necessary to achieve successful regeneration of adult urinary tracts [20, 21, 22].

Stem cells are self–replicating cells that can be used to treat a wide array of clinical conditions in urology [23]. Adult stem cells are located in a specific cellular niche and can be effectively isolated using flow cytometry immunomagnetic–beads–based isolation method or acoustic standing waves [24]. The use of adult stem cells as opposed to human embryonic stem cells for therapy avoids ethical problems and has practical advantages in clinical use. Adult stem cells can be harvested from patients in order to prepare the autologous graft. The risk of tumor formation after adult stem cell transplantation is very low. Mainly embryonic stem cells were reported to form invasive tumors [25, 26].
Bioengineered grafts, composed of biomaterials and stem cells, were proven to induce urinary tract regeneration after implantation. Stem cells are an indispensable tool to modulate the host healing process in order to trigger implanted graft remodeling and further tissue regeneration.
The state of art of stem cells based therapies is still under development. One of the prior issue to research is to identify the most suitable stem cell population for application in reconstructive urology.
Mesenchymal Stem Cells (MSC), Adipose Stem Cells (ASC), Hair Follicle Stem Cells (HFSC), and stem cells derived from amniotic membrane have been evaluated for urinary tract reconstruction [27, 28, 29]. In recent studies, these types of stem cells stimulated urinary bladder regeneration after augmentation with cell–seeded scaffold. However, the authors did not report any significant advantage from the use of a particular type of stem cell. In consequence considering additional benefits, MSCs seem to be the most promising cell source. MSCs are the most often used for experimental cell based therapy in reconstructive urology. Firstly, it is a result of well–established and effective protocols for MSC isolation and *in vitro* propagation [30]. Secondly, the experience gained in the field of hemato–oncology has proven without any doubts that transplantation of MSCs is save and does not bring any risk of tumor formation [31]. Thirdly, MSCs are capable of differentiating into multiple cell types, providing an excellent autologous cell source for cell–based therapy. MSCs differentiate into mesenchymal lineages such as osteoblasts, chondrocytes, and adipocytes [32]. They may also give origin to cell types that do contribute to tissue cell in urinary tract such as urothelial smooth muscle and neuronal cells [33].
Regeneration of smooth muscle cells is crucial for bladder function. In published studies the regeneration of detrusor, which was conducted on hemicystectomy models, was usually partial and did not guarantee proper bladder function [34]. The restoration of a fully organized smooth muscle layer similar to normal has not been reported so far. Regenerated smooth muscle fibers characterize a chaotic arrangement and do not

resemble a highly organized multilayer architecture. Nevertheless, regrowth of detrusor using SIS have been reported in a series of studies to demonstrate normal contractile activity, good innervation, and expression of muscarinic, purinergic, and beta adrenergic receptors in a similar pattern to the intact one [35, 36]. In our recent study that evaluated the function of urinary bladders augmented with amniotic membrane, urodynamic examination revealed bladder motor hyperactivity in most cases [37]. We reached to conclude that regenerated smooth muscle cells created an autonomic cell population that was poorly assimilated to the rest of the urinary bladder wall. This is a result of difficulties to combine smooth muscle regrowth and innervation of regenerated bladder wall.

CONCLUSIONS

Tissue engineering is *cutting edge* health technology and involves the principles of transplantation, materials science, and bioengineering in order to create *de novo* replacement of a diseased or damaged tissue. Tissue engineering efforts have been intensively focused on neo–bladder construction for several decades as a consequence of the increasing number of invasive bladder cancer cases and rising need for new treatment's methods. Alimentary tract segments are commonly used for this purpose, however, this approach is related to many adverse effects. Tissue engineering methods may bring the revolution in this field in the near future. All advances that were done in urological regenerative medicine have provided new technology for urinary bladder wall reconstruction and partial detrusor regeneration for non–malignant disease. Nevertheless, the construction of neo–bladder for patients bearing muscle–invasive bladder cancer is much more challenging due to the inability to use autologous stem cells derived from urinary tracts [38]. This limitation demands searching for new sources of stem cells suitable to be transdifferentiated into smooth muscle cells and urothelial cells. From the other hand, transdifferentiation of stem cells is a controversial process in terms of genomic stability and risk of neoplastic transformation. Before tissue engineering techniques could be recognize as effective and safe for patients, more research studies performed on large animal models and with long follow–up are needed to carry on in the future.

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