Regenerative medicine strategies for treatment of neurogenic bladder

Neurogenic bladder is a general term encompassing various neurologic dysfunctions in the bladder and external urethral sphincter caused by damage or disease. Therapeutic management options fall into the categories of conservative, minimally invasive or surgical. The current standard for surgical management is bladder augmentation using intestinal segments. However, because intestinal tissue possesses different functional characteristics to bladder tissue, numerous complications can ensue. Regenerative medicine uses combinations of cells and/or biomaterials to encourage regeneration of healthy tissue and offers an alternative approach for the replacement of lost or deficient organs, including the bladder. Promising results using the principles of regenerative medicine have already been obtained in children with neurogenic bladder caused by myelomeningocele. Human clinical trials, governed by the US FDA, are ongoing in the USA in both children and adults to further evaluate the safety and efficacy of this technology for regenerating bladders. More studies are in progress and additional advances in this field can be anticipated.

KEYWORDS: bladder replacement ■ neurogenic bladder ■ regenerative medicine ■ stem cell ■ tissue engineering

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Neurogenic bladder is the term used to describe the alterations in bladder function provoked by neurologic dysfunction due to disease or injury. Neurologic bladder dysfunction increases the morbidity of the underlying cause [1]. Diseases at or above the brain stem may cause neurogenic bladder (e.g., cerebrovascular disease, cerebral palsy, Parkinson's disease, traumatic brain injuries and brain tumors). Likewise, diseases involving the spinal cord (e.g., sacral agenesis, tethered spinal cord, traumatic cord injuries, multiple sclerosis and transverse myelitis) may lead to neurogenic bladder dysfunction [2,3]. The most common cause of neurogenic bladder in children is neurospinal dysraphism, more commonly known as spina bifida, which affects one in 1000 newborns [4].

The main goals of treating neurogenic bladder are to maintain renal function by preserving the upper urinary tract, and improving the quality of life by reducing complications. The first goal is accomplished by reducing the intravesical pressure during bladder filling and emptying. The second goal focuses on controlling, or at least minimizing, urinary incontinence and urinary tract infections, which facilitate the patients' social inclusion and rehabilitation [5,6].

Bladder dysfunction associated with neurogenic bladder may manifest in different ways [5]. Urodynamic evaluation is important to define aspects of bladder and sphincter function such as bladder overactivity or underactivity associated with either sphincter synergy or dyssynergy [2]. The most serious situations occur with high intravesical or leaking pressures (above 40 cm H_2O) and detrusor–sphincter dyssynergy, because they represent a potential risk for upper tract deterioration. Children with myelomeningocele and neuropathic bladder, if not managed properly, face a 50% or greater risk for upper urinary tract deterioration [7,8]. The importance of correct diagnosis and therapy for neurogenic bladder patients is illustrated by the fact that between 1914 and 1918, 50% of soldiers in Europe with neurogenic bladder caused by wounds incurred during World War I died. Today's modern technologies for diagnosis and therapy have reduced the mortality rate for neurogenic bladder to less than 3% [9].

Treatment options for managing the urologic symptoms of neurogenic bladder include those that are classified as conservative (e.g., catheterization and pharmacotherapy), minimally invasive (e.g., Botox® injections and neurostimulator implants) and surgical. Surgical management of neurogenic bladder augments the diseased bladder with a bladder dome built from intestinal tissue and is frequently performed in infants to prevent or delay the need for dialysis or kidney transplantation. However,

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use of intestinal segments in the urinary tract carries significant risks, such as excessive mucus production, urolithiasis, metabolic disturbances and malignancy [2,6,10,11].

Regenerative medicine offers potential new therapeutic options for congenital diseases, trauma, cancer, inflammation or other conditions in which organ structure and function are damaged or completely lost [12,101]. In the case of neurogenic bladders, regenerative techniques could be used either locally, at the level of the bladder, or in superordinate centers, such as the CNS. Stem cells, an aspect of regenerative medicine, could have therapeutic value in neurologic disorders [13]. For example, it has been suggested that stem-cell therapy in patients with Parkinson's disease would also treat the neurogenic bladder dysfunctions found in these patients [14]. Since principles of regenerative medicine in neurologic disorders would go beyond the scope of a single review, this article focuses on the basic principles of regenerative medicine and their local applicability in neurogenic bladder.

Basic principles of regenerative medicine

Regenerative medicine offers potential new therapeutic options for congenital diseases, trauma, cancer, inflammation or other conditions in which organ structure and function are damaged or completely lost [12,101]. Regenerative medicine combines the principles of cell biology, materials science and biomedical engineering to fully restore or, at minimum, improve the function of damaged or diseased organs. The introduction of cells is designed to stimulate regeneration, promote vascularization and/or supplement the production of hormones and growth factors. Biomaterials, which include natural and synthetic matrices commonly called scaffolds, are important tools in regenerative medicine. Biomaterials may introduce bioactive factors [15,16] or may attract factors from the body following implantation [17,18]; the objective is to encourage the body's natural ability to repair itself. Biomaterials or combinations of biomaterials and cells can guide the direction of new tissue growth and provide the proper environment to restore tissue or organ structure and function.

At this point in the review, it is necessary to distinguish between regenerative medicine therapy and regenerative medicine products. The first describes prevention, treatment, cure or mitigation of disease or injuries in humans by the administration of autologous, allogeneic or xenogeneic cells that have been manipulated or altered *ex vivo*; the second refers to the process of engineering a tissue in the laboratory, followed by implantation of that tissue into a patient. In this case, the product could consist either of a supporting matrix or a matrix seeded with cells.

Biomaterials

Biomaterials used in regenerative medicine are designed to support restoration of the biological and mechanical properties of native tissues [19,20]. Biomaterials can serve as a carrier for transplanted cells and provide support for structured tissue formation by endogenous cells. Biomaterials can be synthetic, derived from natural substances or a combination of both. Depending on the specific needs, biomaterial configurations can be liquid, gel or solid. Biomaterials can physically control where injected cell suspensions localize in the body. Biomaterials can also provide guidance for appropriate development of neotissues [17,21]. In addition, biomaterials designed as artificial extracellular matrices (ECM) can deliver bioactive signals (e.g., growth factors) to regulate and improve cell function [18] by carrying desired factors into the body and releasing them at a known rate.

Ideally, a biomaterial should be biocompatible, possess similar physical properties to native tissue and promote cellular interaction and tissue development [18–20]. Three different classes of biomaterials are used in regenerative medicine: synthetic polymers (e.g., polyglycolic acid, polyactic acid and polylactic–coglycolic acid [PLGA]), naturally derived materials (e.g., collagen, keratin and alginate) and acellular tissue matrices (e.g., decellularized submucosa from bladder or small-intestinal tissue). Naturally derived materials and decellularized tissue matrices are considered to have biological properties that mimic native tissue or organ ECM (and are thought to elicit fewer immune responses), but are limited in supply and have compositions that are difficult to control or even characterize. On the other hand, synthetic scaffolds can be produced on a large scale with controlled properties of strength, microstructure and degradation rate.

Cells

Cells can be isolated from several sources. Two important aspects to consider in sourcing are the individual and the body location from which the cells are initially isolated [22]. Autologous cells are isolated from the recipient of the eventual regenerative medicine procedure. Cells obtained from any other human being besides

the recipient are allogenic. Cells obtained from nonhuman sources are xenogenic. Cells isolated from the same tissue or organ as the target are homologous and cells isolated from a different tissue or organ are heterologous. Cell sources can be any combination of individual and body location (e.g., autologous homologous, allogenic heterologous and so on).

Cells can be administered in different physical formats: in suspension or attached to a support matrix. The goal of the chosen approach is to restore or repair tissues and organs with the most native outcome and the least complications [12,20,22,23,101].

Autologous homologous cells are a favored choice, because rejection is not an issue (avoiding the need for immunosuppressant therapy) and the cells are re-implanted into a homologous tissue [22]. Nevertheless, autologous homologous cells may not be available for certain applications; one reason is that some cells are difficult to expand to the numbers required, another is a lack of homologous donor tissue due to prior removal or disease (e.g., cancer). Methods for *ex vivo* expansion of a variety of primary human cells have been developed, making the use of autologous homologous cells for some clinical applications a reality, although there are still many opportunities to expand autologous homologous cell-sourcing options.

Another consideration when planning regenerative medicine approaches is the condition of the tissue or organ to be replaced. In some cases, diseased organs may not provide suitable cells for regeneration use, either because of expansion challenges or innate qualities that are incompatible with the type of healing process required. For example, re-introducing malignant cells into a scaffold designed to replace tissue or organs removed for cancer treatment would not be a desirable strategy. However, in some disease states, genetically normal progenitor cells exist in the tissue and are reservoirs for new cell formation [24]. These normal progenitor cells are programmed to give rise to normal tissue, regardless of whether they reside in a normal or diseased environment. Therefore, the tissue- or organ-resident progenitor cell niche and its role in regeneration remains a fertile area of ongoing investigation.

Progenitor & stem cells

Once a source of cells is identified, the cells must be characterized. Two important characteristics are the potential for self-renewal and differentiation [22]. Cells limited in their capacity for self-renewal divide in culture for a finite number of passages. Cells with unlimited self-renewal can be grown in culture for extended times.

Differentiation potential can be unipotent, multipotent or pluripotent. Unipotent cells maintain their phenotype. Urothelial and bladder smooth-muscle progenitor cells isolated from bladder biopsies are unipotent [25,26]. Multipotent cells can be guided into several phenotypes depending on culture conditions, but the range of possible phenotypic outcomes is limited. Adipose tissue and bone marrow are two common sources of multipotent progenitor cells that can be guided into chondrocyte, adipocyte or smooth-muscle differentiation pathways [27–30]. Pluripotent cells have unlimited differentiation potential – they can become any cell type in the body. Embryonic stem cells are pluripotent [27].

Autologous homologous cells are typically progenitor cells and a preferred choice for regenerative medicine [22]. In situations when autologous cells cannot yet be expanded from a particular tissue (e.g., pancreas) or are otherwise unavailable for expansion (e.g., total-bladder replacement for bladder cancer patients), heterologous cells are required.

Pluripotent stem cells are attracting attention from researchers and the public because of their unlimited self-renewal and plasticity of differentiation outcomes; however, their clinical application is limited [27,31]. Pluripotent stem cells are usually allogenic, and thus have the potential to evoke an immune response. Another challenge to using embryonic stem cells clinically is controlling localization and phenotype for the timeframes required for regenerative medicine and tissue-engineering applications. Embryonic stem cells also form teratomas, further complicating their development into clinical products. Developers of products using embryonic stem cells will need to address these characteristics when seeking regulatory approval. Some countries have limited or banned clinical use of embryonic stem cells via legislation.

Multipotent progenitor cells are a potentially beneficial alternative for regenerative medicine and tissue engineering, as their use avoids ethical issues surrounding pluripotent embryonic stem cells. Multipotent progenitor cells can be autologous and have not been observed to differentiate into malignant phenotypes. Fewer nonmesenchymal multipotent cells have been identified to date, and those that are known are present in low numbers and are difficult to maintain and expand. Further research is needed before these cells can be used in clinic.

Recently, stem cells derived from amniotic fluid and placentas have been described [32,33]. Amniotic cells are immuno-selected by their expression of the surface antigen *c-kit* (CD117), the receptor for a protein called stem cell factor [34]. Amniotic pluripotent cells express embryonic markers, such as OCT4 and SSEA-4, but do not form teratomas. These cells have the ability to differentiate into cells of all three embryonic germ layers and have a high self-renewal. Amniotic pluripotent cells represent a new class of pluripotent cells, with features similar to embryonic stem cells, but with potentially fewer obstacles to clinical use.

Cells derived from cloning techniques

Somatic cell nuclear transfer (SCNT) is being investigated as another potential source of pluripotent stem cells for regenerative medicine [35–37]. SCNT involves implanting a nucleus from a donor somatic cell into an enucleated oocyte and generating embryonic stem cells from the union of donor genetic material and oocyte cytoplasm. The resulting cells are genetically identical to the donor. The hypothesis is that these cells can serve as autologous cells for regenerative medicine because of their anticipated potential for stable differentiation into almost any type of cell in the adult body, and thus may be useful in tissue and organ regeneration applications where autologous homologous cells are unavailable [36,38].

SCNT differs from the controversial procedure of reproductive cloning. In reproductive cloning, cells from a SCNT procedure are allowed to develop into a blastocyst that is implanted into a pseudopregnant female, giving rise to an offspring that is genetically identical to the donor of the nucleus. For regenerative medicine, cells from a SCNT procedure are propagated in cell culture and not as implantable embryos. Due to its controversial nature, reproductive cloning is banned for human applications in many countries.

Regenerative medicine applied to the bladder

Current treatment options for bladder augmentation or replacement are fraught with complications, driving the search for new therapeutic approaches [6,11]. The current standard of care uses gastrointestinal segments for bladder replacement or augmentation. However, the nature and function of gastrointestinal mucosa is quite different to bladder wall tissue, resulting in immediate and long-term complications. The bladder must store urine for prolonged periods and serves as a barrier to prevent highly permeable molecules eliminated in urine from returning to the bloodstream. Specialized cells, called umbrella cells, form the permeability barrier and are located in the apical membrane of the urothelial lining of the bladder lumen. Umbrella cells are interconnected by high-resistance tight junctions, which separate the basolateral from the apical cell membranes and block transepithelial ion flux [39,40]. In contrast, gastrointestinal tissues are designed to absorb solutes from the gut lumen into the bloodstream. Therefore, the substitution of gastrointestinal tissue for bladder tissue often leads to metabolic disturbances, urolithiasis, infection, perforation and increased mucus production. Another complication of prolonged exposure of gastrointestinal tissue to the chemical composition of normal urine is malignancy [6,11,41,42]. To avoid these problems, urologists are participating in US FDA human clinical trials to determine the safety and efficacy of regenerative medicine alternatives.

Preclinical experiences with alternatives to enterocystoplasty

Tissue expansion

Progressive dilation of the bladder (and ureters) with an expansion balloon device has been proposed as a method of bladder augmentation, but has not been attempted clinically. In preclinical models, tissue expansion increased bladder capacity between 190 and 380% [43]. Urodynamic assessments and histology of bladder tissue revealed that bladder compliance was in the normal range, as was tissue morphology. Dilated bladder walls maintained normal phenotypic characteristics as shown by immunohistochemistry [44].

Seromuscular grafts

Replacing the traditional gastrointestinal segments used for bladder augmentation with de-epithelialized seromuscular grafts from ileum [45] was another attempted approach. In preclinical models, seromuscular flaps used for bladder augmentation showed normal reepithelialization, but the tissue experienced significant shrinkage that severely limited the clinical feasibility of this technique [46]. The use of such de-epithelialized segments over native urothelium was also attempted but showed little success [47]. Today, the use of seromuscular grafts has been mostly abandoned.

Acellular extracellular matrices & synthetic grafts for bladder repair

Acellular ECM have been used to encourage ingrowth of bladder wall components for partial bladder repair *in vivo*. Typically, these matrices are prepared by mechanical and chemical treatment of native tissue from an allogenic or xenogenic donor to remove all cellular components. Although the matrices are not autologous, antigenicity has not been reported to date [48,49]. Small intestine submucosa (SIS), a xenogenic, heterologous, biodegradable, acellular, collagen-based tissue matrix, was first used in the 1980s for tissue replacement in vascular surgery [50]. In preclinical models of bladder augmentation, SIS elicited bladder wall repair *in vivo* [51]. Bladder repair was shown to be more reliable when SIS was gained from distal ileum [52].

Synthetic and acellular ECM were studied in preclinical models to determine their feasibility as alternatives to enterocystoplasty in patients. Taken together, the results reveal that urothelial regeneration occurs with a variety of graft materials. In contrast, regeneration of the muscle layer of bladder wall has not been observed in large mammals treated with synthetic and acellular ECM grafts; fibrosis and graft contraction of up to 60–70% were consistent outcomes [53–56]. While acellular matrices may have a future role in bladder augmentation and neurogenic bladder treatment, further research is required.

Bladder regeneration in humans

Precursor urothelial and smooth-muscle cells have been isolated and expanded to numbers suitable for regenerative medicine approaches from normal and neurogenic human bladder tissue [25,57–60], establishing the feasibility of using autologous homologous cells in regenerative medicine for bladder augmentation and replacement in humans. In preclinical studies, cell-free and progenitor cell-seeded bladder-shaped scaffolds made from biodegradable polyglycolic acid coated with poly-D-L-lactide-co-glycolide 50:50 (PLGA) were compared [61,62]. Multiple studies have demonstrated that cell-free implants resulted in bladder wall reparative healing (e.g., fibrosis in muscle layer) and that cells were necessary to elicit bladder regeneration [61–63].

Bladder function is assessed with urodynamic measurement of capacity and compliance (the ability of the bladder wall to adjust to urine volume). Bladder structure is assessed by cystoscope or ultrasound to visualize the shape and position of the bladder organ, and histology and immunohistochemistry to visualize tissue formation and composition. Multiple preclinical studies demonstrated that implants with progenitor cells elicited superior structure and function outcomes compared with cell-free implants [61–63]. Furthermore, the cell-seeded implants had long-term durability and were bioresponsive to recipients' needs for bladder capacity [63]. Bioresponsiveness is an important characteristic for clinical applications where the recipient is an infant or young child, because a bioresponsive implant will adjust over time as the recipient grows, just like a native organ.

Regenerative medicine approaches for bladder augmentation following cystoplasty were used in humans for the first time in 1998. In a small pilot study of seven patients, implants made from collagen or PLGA-based scaffolds seeded with autologous progenitor cells were tested [64]. Patients showed increased compliance, decreased end-filling pressures, increased capacities and longer dry periods after implantation. This pilot study established the feasibility and safety of using regenerative medical products as an alternative to gastrointestinal tissue for bladder augmentation. Since this clinical study, the production of progenitor cell-seeded PLGA-based scaffolds for clinical use in augmentation cystoplasty has been standardized and is currently in Phase II clinical trials.

Summary & conclusion

Regenerative medicine approaches are currently being developed for every type of tissue and organ within the urinary system. Most of the effort expended to regenerate genitourinary tissues has occurred within the last decade. A pipeline of regenerative medicine technologies now exists with products in discovery, preclinical testing and clinical trials. Research is underway to expand both cell source and biomaterials options for regenerative medicine applications. Recent progress has established the feasibility of using regenerative medicine to treat neurogenic bladder and the future promises that regenerative medical treatment options will expand to patients with other bladder diseases, and ultimately additional organs and indications.

Future perspective

The results of the two ongoing Phase II clinical studies involving pediatric patients with spina bifida and neurogenic bladder and adult patients with spinal cord injury and neurogenic bladder to evaluate the safety and effectiveness of the Neo-Bladder Augment™ will be

a considerable step in the consolidation of the use of regenerative medicine in the treatment of neurogenic bladder. Future goals are products for urinary diversion and total-bladder replacement (both currently in preclinical good laboratory practice studies), ultimately enhancing or fully restoring bladder function and nerve development.

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Executive summary

- Neurogenic bladder is a general term encompassing various neurologic dysfunctions of the bladder.
- Current surgical management (bladder augmentation using intestine) is burdened by multiple complications due to tissue heterogeneity.
- Regenerative medicine uses combinations of cells and/or biomaterials and offers an alternative for the replacement of deficient organs.

Basic principles of regenerative medicine

- Regenerative medicine combines the principles of cell biology, materials science and biomedical engineering to restore or improve the function of damaged and diseased organs.
- Biomaterials in regenerative medicine provide mechanical support for native tissues, can serve as a carrier for transplanted cells and can deliver bioactive signals (e.g., growth factors).
- Biomaterials can be synthetic, derived from natural substances, or a combination of both.
- Cells for regenerative medicine applications are either obtained from the same individual (autologous), from the same species (allogenic) or another species (xenogenic) and can be expanded, manipulated and/or altered for their specific regenerative purpose.
- Autologous cells are isolated from the same organ they will be used to regenerate. Heterologous cells are obtained from another organ.
- Some cells are difficult to expand or are scarce due to removal of diseased tissue (e.g., cancer). In this scenario stem cells offer a therapeutic alternative.
- Stem cells possess unlimited self-renewal and plasticity. However, stem cells are usually allogenic, and thus have the potential to evoke an immune response.
- Embryonic stem cells can also form teratomas. Researchers using embryonic stem cells will need to address these characteristics when seeking regulatory approval.
- Somatic cell nuclear transfer is another potential source of pluripotent stem cells for regenerative medicine.

Regenerative medicine applied to the bladder

- Current treatment options for bladder augmentation or replacement are fraught with complications, driving the search for new therapeutic approaches.

Preclinical experiences with alternatives to enterocystoplasty

- Progressive dilation of the bladder with a balloon showed good preliminary results in animal models, but has not been attempted clinically.
- Seromuscular flaps showed normal re-epithelialization, but the tissue experienced significant shrinkage. Today, the use of seromuscular grafts has been mostly abandoned.
- Acellular extracellular matrices and synthetic grafts for bladder repair have been investigated.

Bladder regeneration in humans

- Promising results using cell-seeded biodegradable scaffolds have been obtained in children with neurogenic bladder caused by myelomeningocele.
- Human clinical trials, governed by the US FDA, are ongoing in the USA to further evaluate the safety and efficacy of these regenerative technologies.

Summary & conclusion

- Regenerative medicine technologies now exist with products in discovery, preclinical testing and clinical trials.
- Recent progress has established the feasibility of using regenerative medicine to treat neurogenic bladder.
- Regenerative medical treatment will expand to patients with other bladder diseases, and ultimately additional organs and indications.

Future perspective

- Future goals are products for urinary diversion and total bladder replacement (both currently in preclinical good laboratory practice studies).

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▪ **Consensus on further development.**