

Review Article

Cell-Based Therapy for Urinary Incontinence

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Urinary incontinence has become a societal problem that affects millions of people worldwide. Although numerous therapeutic modalities are available, none has been shown to be entirely satisfactory. Consequently, cell-based approaches using regenerative medicine technology have emerged as a potential solution that would provide a means of correcting anatomical deficiencies and restoring normal function. As such, numerous cell-based investigations have been performed to develop systems that are focused on addressing clinical needs. While most of these attempts remain in the experimental stages, several clinical trials are being designed or are in progress. This article provides an overview of the cell-based approaches that utilize various cell sources to develop effective treatment modalities for urinary incontinence.

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INTRODUCTION

Urinary incontinence, which is characterized as any involuntary leakage of urine, is a social and distressing problem that affects approximately 25 percent of women and 9 percent of men [1,2]. Although urinary incontinence is classified by a variety of types, the underlying pathology can be grouped into two major categories: urethral hypermobility and intrinsic sphincter deficiency [3]. However, most cases of urinary incontinence exist between the extremes of these two categories, and most patients have elements of both disorders [4]. Although conservative management, such as dietary and lifestyle changes, pelvic floor muscle training, and pharmacologic agents, has been somewhat helpful in alleviating symptoms in mild conditions, these measures do not correct the underlying pathology [5-9]. In contrast, surgical and interventional procedures seek to increase the coaptation pressure of the urethral sphincter by means of sling procedures, artificial sphincter placement, and injectable bulking agents [10]. Sling procedures are considered the gold standard in correcting the underlying anatomical deformities, with success rates ranging from 80% to 94% [11-13]. However, complications associated with these procedures and long-term consequences of urethral and vaginal erosions of the sling materials remain as unsolved challenges. Injectable bulking agents, such as polytetrafluoroethylene, bovine collagen, silicone particles, and carbon beads, have also been ap-

plied clinically as a treatment modality for incontinence. These agents are designed to augment urethral and bladder neck tissues by increasing resistance to urine flow, but they have minimal effects on urethral mobility. As such, approximately 25% of patients with incontinence show correction of urinary incontinence, whereas 50% demonstrate improvement and the remaining 25% experience treatment failure [14,15]. More importantly, untoward effects associated with the bulking agents, including long-term biocompatibility, recurrence, inflammatory responses, migration, and erosion, remain a problem [11,16].

Due to the limitations of the current treatment modalities for urinary incontinence, investigators have sought alternative approaches to treating urinary incontinence. Regenerative medicine has emerged as an innovative scientific field that focuses on the development of new approaches to repairing cells, tissues, and organs for clinical applications. Recent advances in cell-based technology using regenerative medicine techniques suggest that this approach holds enormous potential to improve human conditions by encompassing alteration of the current biological state of a targeted tissue, augmentation of depleted function, or absolute functional tissue replacement [17-19]. Consequently, efforts in cell-based technology have focused on developing systems that would restore and maintain normal tissue function. To that end, numerous cell-based investigations have been performed to address urinary incontinence. This article aims to provide an overview

of the cell-based investigations that utilize a wide range of cell sources to overcome the limitations of current treatment modalities for urinary incontinence.

FUNDAMENTALS OF CELL-BASED THERAPY FOR URINARY INCONTINENCE

The basic components required to achieve functional tissues and organs are cells, scaffolds, and the *in vivo* environment [17,18]. Regenerative medicine strategies that have been demonstrated to be successful involve the use of biocompatible matrices either with or without cells. The matrices are used either as supporting scaffolds to promote and facilitate tissue regeneration over smaller gaps or as cell delivery vehicles for larger defects. When cells are used, donor tissue is dissociated into individual cells, which are expanded in culture, combined with a support matrix, and introduced into the body to form mature and functional tissues. The cells can be delivered into the target region surgically or through a needle injection, depending on the type of tissue applications. Cells for tissue reconstitution can be derived from the native organ to be replaced, thus avoiding rejection. In instances where normal tissues are not available, different cell sources may be explored. Stem and progenitor cells offer numerous opportunities in regenerative medicine. Cells derived from various stages of development can be either implanted directly in the target tissues or guided into specific cell lineages *in vitro* followed by implantation *in vivo*.

Cells are an essential component required to augment tissue function for urinary incontinence. Cells derived from various sources have been used for urinary incontinence, including chondrocytes, smooth muscle cells, muscle precursor cells (MPCs), adipose-derived stem cells, and bone marrow stromal cells [20]. The use of these cells is aimed at achieving coaptation of the bladder neck region by augmenting tissue mass or restoring sphincter function. To use cells for application in urinary incontinence, a cell expansion system that permits recovery of large quantities of target cells must be developed. This has been one of the challenges that is being continuously investigated. Even when some organs, such as the liver, have a high regenerative capacity *in vivo*, *in vitro* expansion of cells derived from these organs has proved to be difficult. However, the discovery of privileged sites for committed precursor cells in specific organs and extensive study of the conditions that promote precursor cell maintenance and differentiation within these sites have begun to overcome some of the limitations associated with cell expansion *in vitro*. Urothelial cells, for instance, have been grown in culture in the past, but with only limited success. However, several novel culture protocols have been developed over the past two decades that allow for the maintenance of precursor cells in an undifferentiated state. Because these cells can remain in the growth phase, the ability to expand urothelial cultures is vastly improved [21-24]. These studies indicate that it may be possible to collect autologous cells

from patient's tissues, expand them in culture, and return them to the donor in sufficient quantities for reconstructive purposes [23,25-30].

Biomaterials for various cell-based therapies are usually designed to replicate the biological and mechanical functions of native tissue structures and their extracellular matrix (ECM). They provide three-dimensional architecture for cells to reconstitute into new tissues and allow for the delivery of cells and bioactive factors (e.g., peptides, growth factors) to desired sites in the body to enhance functionality [31]. Because most mammalian cell types are anchorage-dependent, biomaterials serve as a cell-adhesion substrate that can deliver cells to specific regions of the body with a high loading efficiency. An ideal biomaterial for cell therapy should be biodegradable and absorbable without eliciting inflammatory responses that interfere with cellular function and tissue formation. Incompatible materials are destined for an inflammatory or foreign-body response that eventually leads to rejection or necrosis. Because biomaterials for cell therapy provide temporary mechanical support while the cells undergo spatial tissue organization, a suitable biomaterial should maintain adequate mechanical integrity to support tissue formation during early stages of development. To develop a suitable biomaterial for the cell-based treatment of urinary incontinence, various factors need to be considered, including biocompatibility, biodegradation, and structural and mechanical properties. These materials should be able to 1) facilitate the delivery of cells to target sites in the urethra and bladder neck region, 2) maintain the three-dimensional architecture that permits formation of new tissues, and 3) guide the development of new tissues with appropriate function [31]. As such, numerous biomaterials, both synthetic and naturally derived, have been developed and used as substrates for urinary incontinence, including collagen and alginate [32-37].

To achieve functional tissue *in vivo*, implanted cells must maintain viability by obtaining an adequate supply of nutrients and oxygen. One of the continued challenges in engineering clinically relevant tissues is the establishment of vascularization for implanted tissues and organs. This is especially true for large tissue masses consisting of cell-based implants that require a sufficient blood supply to maintain viability. It is well known that cellular implants that are more than a few hundred microns away from a blood vessel will not survive because of diffusion limitations. Consequently, tissue implants that are unable to establish vascularization in time result in cell death and tissue necrosis [38]. This has been a critical limiting factor for developing functional tissues for human applications [39]. An approach to maintaining tissue viability *in vivo* is to place the engineered tissue adjacent to a heavily vascularized tissue such as the omentum to achieve adequate vascularization [40]. However, this approach may not always be feasible because the target implantation site may not be in close proximity. To overcome this limitation, several strategies have been used to facilitate the delivery of

oxygen and improve the survival of implanted cells. Angiogenic growth factors such as vascular endothelial growth factor (VEGF), which is a potent endothelial cell-specific mitogen, have been used to promote neo-vascularization [41,42]. Although the effectiveness of an enhanced angiogenic response has been demonstrated in many tissue systems, the rate of angiogenesis cannot be accelerated, thus limiting the size of implantable tissue masses [43]. Another approach to overcoming diffusion limitation is to configure biomaterials that facilitate vascularization to implanted cells and tissues by modifying properties and porosity [44,45]. Recently, efforts have been placed in developing strategies to prolong cell survival until host neovascularization is achieved by increasing oxygen tension or incorporating oxygen-generating biomaterials [46,47].

CELL-BASED APPROACHES FOR URINARY INCONTINENCE

1. Injectable chondrocytes

One of the early investigations of cell therapy for urinary incontinence involved the use of autologous chondrocytes (cartilage cells). Chondrocytes were chosen as a tissue bulking agent because this cell type possesses an inherent ability to produce extracellular matrix and maintain structural integrity *in vivo*, thus providing ideal tissue bulking for the treatment of urinary incontinence [32]. In addition, chondrocytes can be easily isolated, grown, and expanded in culture conditions. Alginate, a liquid solution of glucuronic and mannuronic acid, serves as a substrate for the delivery of chondrocytes through a needle. In a preclinical study demonstrating the feasibility of using chondrocytes as a bulking agent, autologous chondrocytes from porcine ear cartilage were grown and expanded in culture. The chondrocytes, suspended in alginate, were then injected endoscopically to correct anatomical deformities of the vesicoureteral junction. This study showed that chondrocytes combined with alginate *in vitro* can be easily injected cystoscopically, and that the elastic cartilage tissue formed within the injection region can correct vesicoureteral reflux without any evidence of obstruction [48].

Based on the results of multiple experimental studies, two multicenter clinical trials were conducted using the engineered chondrocyte technology. In one study, patients with vesicoureteral reflux were treated at 10 centers across the United States by following the same strategy that involved the use of autologous chondrocytes combined with alginate. The patients had a success rate similar to that with other injectable substances in terms of cure [49]. In the second study, patients with urinary incontinence were treated endoscopically with injected chondrocytes at three different medical centers in the United States. Thirty-two patients received a single outpatient injection just distal to the bladder neck. The investigators concluded that this treatment was safe, effective, and durable, with 50% of patients remaining completely dry 12 months after a single

injection. Twenty-six of 32 patients reported at least some improvements at 3 months after the injection that continued until at least the 12-month follow-up visit [32].

2. Injectable muscle cells

The potential use of injectable cultured myoblasts for the treatment of stress urinary incontinence has been investigated [50,51]. Labeled myoblasts were directly injected into the proximal urethra and lateral bladder walls of athymic mice with a micro-syringe in an open surgical procedure. Tissue harvested up to 35 days after injection contained the labeled myoblasts, as well as evidence of differentiation into regenerative myofibers. This study showed that a significant portion of the injected myoblast population persisted *in vivo*. Similar techniques of sphincter-derived muscle cells have been used for the treatment of urinary incontinence in a pig model [52]. The fact that myoblasts can be labeled and survive after injection and begin the process of myogenic differentiation supports the feasibility of using cultured cells of muscular origin as an injectable bioimplant.

The use of injectable MPCs has also been investigated for use in the treatment of urinary incontinence due to irreversible urethral sphincter injury or developmental defects. MPCs are the quiescent satellite cells found in each myofiber that can proliferate to form myoblasts and eventually myotubes and new muscle tissue. The standard MPC grafting technique consists of an injection of cells that were originally harvested from a muscle biopsy by enzymatic digestion and then cultured under specific conditions to eliminate nonmyogenic cells. However, there is now increasing evidence that these successive steps of MPC preparation alter the myogenic potential of these cells *in vivo* [53,54].

MPCs have been shown to play an active role in the regeneration of injured striated urethral sphincter [55]. In a subsequent study, autologous MPCs were injected into a rat model of urethral sphincter injury, and replacement of mature myotubes as well as restoration of functional motor units were noted in the regenerating sphincter muscle tissue [56]. This was the first demonstration of the replacement of both sphincter muscle tissue and its innervation by the injection of MPCs, which suggests that MPCs may be a minimally invasive solution for urinary incontinence in patients with irreversible urinary sphincter muscle insufficiency. Another study investigated a method of intraurethral MPC transplantation consisting of implanting freshly isolated myofibers with their satellite cells. The investigators hypothesized that myofiber death after implantation would induce activation of satellite cells *in vivo*, thus avoiding the necessity for MPC extraction and *in vitro* expansion [57]. Histological studies showed that the myogenic process, consisting of myofiber degeneration and satellite cell activation, was observed at 7 days after transplantation, followed by myotube formation replacing parental myofibers at 30 days. In the subsequent experiments, implantation of myofiber strips to endoscopic sphincter injured urethra generated a pressure peak that

decreased after endoscopic injury and reappeared 60 minutes later, indicating that this action was tonic and under neural control. Histologically, the myotubes were oriented in the same direction as the parental myofibers, suggesting that satellite cell fusion was guided by the tubes of extracellular matrix surrounding each myofiber. There was evidence that new nerve fibers developed in the vicinity of the implanted myotubules, and neural tissue was present in greater density than in surrounding tissue. These findings suggest the presence of concomitant neuronal development and innervation of the transplanted myofibers. In contrast, the experimental results of another animal study indicated that the improvement of muscle regeneration was not better than that with muscle-derived stem cells [58]. The reasons for this discrepancy remain to be explored.

Cultured myoblasts are one of the most studied cell types for the treatment of incontinence [59,60]. The fact that MPCs survive after injection and initiate the process of myogenic differentiation further supports the feasibility of using cultured cells of muscular origin as an injectable bioimplant [56]. Although many studies have been performed in laboratory settings using protocols developed for animal cells to demonstrate the principle and feasibility of using cultured cells of muscular origin, culture systems compatible with clinical application are needed [61,62]. One problem in these established protocols is the use of extracellular matrix proteins derived from cancer cells (Matrigel[®], BD Biosciences, San Jose, USA), which contain a high concentration of various growth factors and are commonly used to promote the proliferation and differentiation of muscle precursors. In order to achieve rapid clinical translation of MPC therapy, culture conditions were optimized for human application. A human MPC culture system that uses FDA-approved substrates with defined media for expansion and differentiation was developed [62].

An interesting clinical trial that used striated muscle-derived myoblast transplantation was reported by Strasser et al [63]. Using transurethral ultrasound-guided injections of autologous myoblasts and fibroblasts, 42 patients (29 women, 13 men) with urinary stress incontinence were treated. In 35 patients, urinary incontinence was completely cured. In seven patients who had undergone multiple surgical procedures and radiotherapy, urinary incontinence improved but was not eliminated. No side effects or complications were reported postoperatively. In this study, the authors concluded that urinary incontinence can be treated effectively with autologous stem cells. It is noteworthy that this represents the first attempt to use an autologous stem cell strategy in clinical urology. The same investigators conducted a randomized controlled trial comparing ultrasound-guided transurethral rhabdosphincter injection of autologous myoblasts and fibroblasts vs. transurethral collagen injection for incontinence in 63 eligible women [64]. At the follow-up examination at 12 months, 38 of the 42 women injected with autologous cells were completely continent, compared with 2 of the 21 patients given conventional treatment with collagen.

However, this report was later retracted by the Lancet because of several controversial issues [65]. Despite the retraction of the report on this clinical trial, several other FDA-approved cell therapy clinical trials targeting incontinence are on the horizon (<http://www.clinicaltrials.gov>).

3. Stem cells

One of the objectives of regenerative medicine therapy for incontinence is reconstruction of the sphincter muscle itself. Stem cells have been proposed as a promising cell source to replace, repair, or enhance the biological functions of damaged sphincter [66]. Stem cells are defined by their ability to self-renew and differentiate into a variety of cell types. They are further classified by the breadth of cell lineages into which they may potentially differentiate [67]. Research that explores the possible applications of stem cells in the field of urology has been increasing. The current strategy for the cell-based approach to build functional tissues uses autologous cells from the diseased organs. However, in instances where normal cells cannot be obtained because of extensive end-stage disease, stem cells are envisioned as an ideal source of cells because they can differentiate into desired cell types if guided appropriately [68].

The pluripotency of human embryonic stem cells is highlighted by their ability to form embryoid bodies *in vitro*, which are cell aggregations that contain all three germ layers. The use of embryonic stem cells as a primary non-immunogenic tissue source for seeding of decellularized scaffolds has been heavily investigated [69]. However, clinical applications using embryonic stem cells are faced with several challenges, including the propensity of these cells to form teratomas *in vivo* and ethical concerns about the destruction of human embryos [70]. Recently, fetal stem cells derived from amniotic fluid and placenta have been described and represent a novel source of stem cells [71,72]. These cells express markers consistent with human embryonic stem cells, such as OCT4 and SSEA-4, but they do not form teratomas. The cells are multipotent and can differentiate into cells from all three germ layers. In addition, the cells have high replicative potential and can be stored for future self-use, without the risks for rejection and without ethical concerns [73]. Although these cells do differentiate into the myogenic lineage, further studies are necessary to determine their utility in urinary incontinence applications.

Multipotent stem cells have become an attractive option for stem cell therapies in many organ systems, including the urologic system. The use of these cells avoids the controversy surrounding human embryonic stem cells, and unlike embryonic cells, they do not trans-differentiate into a malignant phenotype. Thus, there is a diminished risk for teratoma formation when implanted *in vivo* and they can be extracted from many different tissues, including bone marrow, striated muscle, fat, skin, testicle, and synovial membrane [74]. Adult-derived stem cells, such as adipose-derived stem cells and bone marrow stromal cells or

mesenchymal stem cells (MSCs), are gaining popularity because they have a more extensive differentiation potential than previously reported [75-77]. One limitation that hampers rapid clinical translation is the expandability of the cells. Strategies to overcome this limitation are being developed, such as adding growth factors to the injected cells to stimulate proliferation or culturing cells on biodegradable matrices composed of natural extracellular matrix proteins or synthetic polymers [78-80].

The use of adipose-derived stem cells has been explored for the treatment of urinary incontinence [81]. Processed lipoaspirate (PLA) cells are abundant in the adipose tissue, and they are amenable to harvesting under local anesthesia. These cells are phenotypically similar to mesenchymal stem cells and have been shown to differentiate into adipogenic, chondrogenic, osteogenic, neurogenic, and myogenic lineages [82]. In one study, lipoaspirates from female patients undergoing liposuction were processed to yield a pluripotent population of PLA cells that were injected into the bladder and urethra. Eight weeks after injection, PLA cells demonstrated the expression of alpha-smooth muscle actin, an early marker of smooth muscle differentiation. This study suggests that PLA cells are an easily accessible source of pluripotent cells, making them ideal for tissue regeneration. Human PLA cells injected into the urinary tract show morphological and phenotypic evidence of smooth muscle incorporation and differentiation over time. In another study, the effects of adipose-derived stem cells isolated from the peri-ovary fat were implanted in a rat model of incontinence [83]. This study showed that the treated animals showed a normal voiding pattern and possessed higher elastin and smooth muscle content than did untreated incontinent animals.

Bone marrow derived MSCs have been used as a cell source for the treatment of urinary incontinence [84]. MSCs were exposed to 5-azacytidine to induce myogenic differentiation and were culture expanded. Subsequently, these cells were implanted into the bladder neck region of athymic rats. The results of this study suggested that the use of MSCs for the treatment of urinary incontinence may be a feasible option [84].

CONCLUSIONS

Rapid advances in cell-based technologies have provided innovative therapeutic opportunities for numerous human conditions. This article presents cell-based approaches that are designed to address the challenges involved with the current treatment modalities for urinary incontinence. These include the use of a wide spectrum of cell sources ranging from nonurologic autologous cells to stem cells at various stages of development. Although the current status of research in this area remains mostly in the laboratory, several technologies are currently being developed and validated clinically. These innovative developments have generated enormous hope and anticipation that the new modalities will provide effective treatments

and improve the quality of life of millions of patients in the near future.

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