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Mesenchymal Stem Cells for Cell Therapy and Tissue Regeneration in Urology

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1. Introduction

Adult stem cells are defined as clonogenic, self-renewing progenitor cells that reside in adult tissues and can generate one or more specialized types of cells required for the function of that tissue. The majority of adult tissues contain their own stem/ progenitor cells that are capable of maintaining, generating, and replacing terminally differentiated cells within the tissue in response to physiologic cell turnover or tissue damage resulting from injury. Stem cell populations in the bone marrow were the first adult stem cell populations to be described, but in recent years adult stem populations have been demonstrated in the brain,[1, 2] skin, [3] and muscle [4], hair follicle and the gastrointestinal tract. Adult stem cells, especially hematopoietic stem cells, are the best understood cell type in stem cell biology [5], yet they remain an area of intense study, as their potential for therapy may be applicable to a myriad of degenerative disorders. These cells are a particularly attractive option for cell therapy and tissue engineering applications because they can be used in autologous therapies, thus avoiding any complications associated with immune rejection.

Research into adult stem cells has progressed slowly in the past, mainly because true stem cells are present in extremely low numbers in adult tissue[6-8], and because adult non-mesenchymal stem cells have been challenging to isolate, expand and maintain in culture. Some cells, such as those of the liver, pancreas and nerve, have very low proliferative capacity *in vitro*, and the functionality of some cell types is reduced after the cells are cultivated. These issues have limited the use of adult stem cells in tissue engineering and cell therapy research. However, the discovery of native targeted progenitor cells has allowed some of these limitations to be overcome. Native targeted progenitor cells are tissue specific unipotent cells derived from most organs. These cells are already programmed to become a specific cell type, and as with adult stem cells, native progenitor cells can be obtained from the specific organ to be regenerated, expanded, and used in the same patient without rejection, in an autologous manner [9-26]. By studying the niche in which the progenitor cells reside, as well as by exploring conditions that promote the differentiation of these cells, it has been possible to overcome some of the problems facing cell expansion *in vitro*. Major advances in cell culture techniques have been made within the past decade, and these techniques make the use of autologous cells possible for clinical application. In this

chapter, we will focus on the use of autologous cells for regeneration and/or repair of the urinary tract.

2. Stem / progenitor cells derived from the genitourinary tract

Bladder

Adult human stem/progenitor cells from urinary tract system have been recently described and characterized [6,27]. It has been shown experimentally that the bladder neck and trigone area have a higher density of urothelial progenitor cells [28], and these cells are localized in the basal region [29]. In the past, it was possible to grow urothelial cells in the laboratory setting, but only with limited success. However, several protocols have been developed over the last two decades that have improved urothelial growth and expansion by enhancing culture conditions to support proliferation and differentiation of urothelial progenitor cells [17, 30-32]. It is now possible to expand a urothelial strain from a single surgical specimen that initially covers a surface area of 1 cm^2 to one covering a surface area of 4202 m^2 (the equivalent area of one football field) within 8 weeks[17]. Now, normal human bladder epithelial and muscle cells can be efficiently harvested from surgical material, extensively expanded in culture, and their differentiation characteristics, growth requirements, and other biologic properties can be studied [17, 19, 20, 31-40]. In addition, human urothelial and muscle cells can attach and form sheets of cells when seeded onto polymer scaffolds. The cell-polymer scaffold can then be implanted for repairing urological tissue defects. Histological analysis indicates that, within the cell-polymer construct, viable cells are able to self assemble back into their respective tissue types, and they retain their native phenotype [11].

To determine whether these engineered tissues could be implanted in continuity with the urinary tract, large animal models of bladder augmentation were used [22]. Partial cystectomies were performed in dogs and the animals were divided into 2 experimental groups. In one group, the bladder was augmented with a non-seeded bladder-derived collagen matrix, and in the second group, the bladder was augmented with a cell-seeded construct. The bladders augmented with seeded matrices demonstrated a 100% increase in capacity compared with bladders augmented with cell-free matrices, which only generated a 30% increase in capacity.

It has been known for a number of years that the bladder is able to regenerate generously over cell-free scaffolds, because urothelium has a high reparative capacity [41]. However, bladder muscle tissue is less likely to regenerate in the same fashion as urothelium, which leads to contracture or resorption of the graft. In addition, the inflammatory response toward the materials used to form the graft matrix may contribute to the resorption of cell-free grafts as well. The dog study demonstrated a major difference between matrices used with autologous cells (tissue-engineered matrices) and those used without cells [22]. The matrices seeded with cells prior to use in bladder augmentation retained most of their implanted diameter, as opposed to the matrices implanted without cells, in which graft contraction and shrinkage occurred. As in previous studies, the histomorphology demonstrated a lack of muscle cells and a more aggressive inflammatory reaction in the unseeded matrices.

The results of initial studies showed that the creation of tissue engineered bladders using autologous urothelial and smooth muscle cells could be achieved; however, it could not be determined whether the improvement in functional parameters noted was due to the implanted segment or to the remaining native bladder tissue. To better address the

functional parameters of tissue-engineered bladders, additional animal model of subtotal cystectomy with subsequent replacement with a tissue-engineered organ was created[25]. In this model cystectomy-only and non-seeded controls maintained average capacities of 22% and 46% of preoperative values, respectively. An average bladder capacity of 95% of the original precystectomy volume was achieved with the cell-seeded tissue engineered bladder replacements. These findings were confirmed radiographically. The subtotal cystectomy reservoirs that were not reconstructed and the reservoirs reconstructed with unseeded grafts showed a marked decrease in bladder compliance (10% and 42% total compliance, respectively). In contrast, the compliance of the tissue-engineered bladders showed almost no difference from preoperative values (106%). Histologically, the non-seeded scaffolds resulted in a structure composed of normal urothelial cells with a thickened fibrotic submucosa and a thin layer of muscle fibers. The retrieved tissue-engineered bladders showed a normal cellular organization, consisting of a trilayer of urothelium, submucosa, and muscle. Immunocytochemical analyses confirmed the muscle and urothelial phenotypes, and indicated the presence of neural structures [25]. These studies, performed with polyglocolic acid based-scaffolds, have been repeated by other investigators, showing similar results in large numbers of animals long-term [42, 43]. Subsequent studies indicated that biodegradable scaffolds seeded with cells can be used without concerns for local or systemic toxicity [44].

Bladder tissue engineered using autologous cells has been used clinically. A small clinical study was conducted starting in 1998. Seven patients were treated using a collagen scaffold seeded with cells taken from biopsies of their own bladders, either with or without omental coverage, or a combined PGA-collagen scaffold seeded with cells and omental coverage. The patients reconstructed with the engineered bladder tissue created with the PGA-collagen cell-seeded scaffolds with omental coverage showed increased compliance, decreased end-filling pressures, increased capacities and longer dry periods over time [45]. It is clear from this small study that the engineered bladders continued their improvement with time, suggesting continued development in vivo. Although the experience is promising, it is just a start and the technology is not yet ready for wide dissemination, as further experimental and clinical studies are required.

Kidney

Kidney has long been considered an organ that is incapable of true regeneration. Furthermore, the question of whether or not the kidney contains adult stem cells remains controversial. However, increasing evidence of a regenerative response in the kidney has been observed following the injury resulting from both toxic and ischemic insults. These observations include evidence of renal progenitors of specific cell types involved in the formation of new renal tubular cells and the recovery of renal function recovery after ischemic injury. The presence of injured or dead cells following ischemia causes denudation of the tubular basement membrane, and sloughed cells and cellular debris fills tubular lumens. The kidney responds to the ischemic injury with a prompt regenerative response, resulting in regenerating tubules and improving kidney function. Although they remain elusive, the cells participating in renal regeneration are likely from pools of both exogenous and endogenous stem cells. The exogenous stem cells are probably largely derived from bone marrow, and may be both hematopoietic and mesenchymal stem cells. In some studies, these cells appear to home to damaged sites in the injured kidney and form tubular epithelial cells following acute renal injury [46-49]. These MSC might also produce growth factors such as IGF-1 to promote renal repair [50]. The endogenous stem cells are resident

kidney stem cells found in the renal tubules and the papilla[51, 52]. They are inactivated under physiological conditions. These stem cells posses the capacity to give rise renal tubule cells following injury repair [53, 54]. A recent study showed that repopulation of damaged renal tubules occurs primarily from proliferation of tubular epithelial cells and resident renal-specific stem cells, with some contribution of paracrine factors from bone marrow-derived mesenchymal stem cells [55-56].

Testis

Primordial germ cells (PGC) are the embryonic progenitor cells of the gametes (spermatogonial stem cells and ova). *In vivo*, PGC colonize the gonadal ridge during early embryonic development and are then restricted to producing the gametes. However, if PGC are cultured *in vitro* in the presence of specific growth factors, they are able to form pluripotent embryonic germ cells (EGC) through a process that is relatively similar to that of nuclear reprogramming and generation of induced pluripotent (iPS) cells in the laboratory (discussed later in this review) [57]. These cells can contribute to all cellular lineages in chimeric embryos, including the germline. They also form teratomas when injected into immunocompromised animals.

For regenerative medicine purposes, PGC are not ideal, because they are derived from embryos and there are a number of controversial ethical issues surrounding the manipulation of human embryos. However, there has been much interest in isolating and describing spermatogonial stem cells (SSC) in recent years. The presence of SSC, which are derived from PGC in the testis, was originally inferred from the presence of ongoing spermatogenesis in the adult male. Recently, the cells believed to be the actual SSC were isolated from the adult testis of both mice and humans through selection of the markers STRA-8, GPR125, CD49fm, CD133 and others[58, 59 60]. Interestingly, when SSC from both mice and humans are cultured in specified media containing growth factors known to be required for maintenance of pluripotency of other types of stem cells, such as leukemia inhibitory factor (LIF) and glial cell line-derived neurotrophic factor (GDNF), they appear to convert to an embryonic stem (ES) cell like state.[60] These converted cells have been termed adult germline stem cells (aGSC) and they can differentiate into a number somatic cell types encompassing all three embryonic germ layers when they are exposed to the same conditions used to differentiate ES cells. They also form teratomas when implanted *in vivo*. These results suggest that SSC, which can be obtained through a small testicular biopsy, may be useful for the development of cell-based, autologous organ regeneration strategies. However, more research is required to overcome additional hurdles before this technology can be used clinically. In addition, since autologous regeneration strategies based on SSC would only benefit males, researchers are working to identify and describe similar pluripotent cells that may reside in an ovarian niche for use in females.

Urine

We recently demonstrated that it is possible to isolate and expand stem/progenitor cells from human urine[6]. Approximately, 0.2% of cells collected from urine express markers characteristic of mesenchymal stem cells (MSC), can expand extensively in culture, and can differentiate towards multiple bladder cell lineages as identified by the expression of urothelial, smooth muscle, endothelial and interstitial cell markers. We initially referred to these cells as urine progenitor cells. However, our more recent experiments indicated that urine-derived cells can give rise to additional specialized types, including osteocytes,

chondrocytes, and adipocytes. Furthermore, these cells have self-renewal capability consistent with stem cells. There is now sufficient evidence to provisionally designate them as urine-derived stem cells (USC).

Three types of cells exist in urine: differentiated, differentiating and progenitor cells. Most cells in urine are fully differentiated. They do not attach to tissue culture plates. About 0.1% of cells in urine are differentiating cells, which do attach to plates and display the morphology and protein markers of various bladder cell lineages. However, these cells do not expand further after subculture. About 0.2% of the cells in urine have a phenotype consistent with multipotent stem cells. USC are easily cultured, appear genetically stable after a number of passages, and maintain the ability to give rise to more differentiated progeny.

USC comprise an average of about 7 cells/100ml urine (from 5 to 10 cells/100 ml urine). We have shown that, a few days after being placed in a tissue culture well, a single cell forms a cluster of cells which appeared small, compact and uniform. A consistently high yield of cells was achieved from each of these clonal lines. The cells reached confluence in about two weeks when placed in a 3-cm diameter well at passage one. At passage 2, cells were plated in 10 cm culture dishes and a cell number of approximately one million was reached in 3.5 weeks. Finally, in six to seven weeks, the cultures expanded to approximately 100 million cells at passage four. These cells displayed normal exponential cell growth patterns, with a steady increase in cell numbers during a 10-day culture period. The average population doubling time was 31.3 hours in mixed media. These urine-derived cells also showed the ability to differentiate into various cell lineages as described below, and were capable of growing for at least 14 passages *in vitro*.

Cells from human urine specimens could be consistently cultured long-term using a medium that we originally developed for culture of rat urothelium[61]. However, the phenotype of the cultured human urine-derived cells was not that of primary urothelial cells. The primary cultures from urine did not show expression of the cytokeratins (CK7, CK13, and CK19/20), which are characteristic of epithelial cells, nor did the cells express the urothelial-specific protein uroplakin. After growth in medium containing higher levels of epidermal growth factor (EGF), the cells were induced efficiently to express the cytokeratin proteins and uroplakin[6]. However, after growth in myogenic medium, the cultured cells expressed markers consistent with smooth muscle, including alpha-smooth muscle actin (α-SM actin), desmin, calponin, and myosin[6]. This led us to conclude that the urine-derived cells were progenitors (initially designated UPC) capable of giving rise to both urothelium and bladder smooth muscle. Furthermore, we found that the cells displayed a surface marker phenotype consistent with mesenchymal stem cells (MSC). Specifically, they expressed CD 44, CD73, CD90, CD105, and CD 146, and they were negative for both hematopoietic markers and endothelial markers including CD45, CD34, and CD31. We concluded that the urine-derived progenitors were at least bipotential for the major bladder cell types. This result was surprising, because it was generally believed that muscle and epithelial cells in bladder represent separate cell lineages derived from mesoderm and endoderm, respectively. We have recently observed that the urine-derived cells express markers typical of mesenchymal stem cells (MSC) and pericytes, and that they can differentiate to yield the characteristic cell lineages obtained from MSC, namely, osteocytes, adipocytes and chondrocytes [62].

In our recent study, USC clones could be obtained from 85% of urine sample tested. Fresh urine gave the highest rate of colony formation (67%) and urine stored at 4° C the lowest

(30%). Urine from volunteers aged 13-40 gave the highest rate of clone recovery. Catheterization significantly enhanced the number of USC in urine compared to spontaneously voided urine, possibly because catheterization resulted in cells being scraped off the inner bladder wall. Collecting triple urine samples increased the rate of clone formation.

There are many potential advantages to using USC as a cell source for urological tissue engineering. First, cells can be easily harvested and grown in culture. USC do not require enzyme digestion or culture on a layer of feeder cells to support cell growth. Second, since invasive surgical biopsy procedures are not necessary to harvest cells from urine, patient morbidity and potential complications, such as urethral or bladder trauma and urinary tract infections, are avoided. As USC are autologous somatic cells, no ethical issues are involved in their use for tissue reconstruction, and no immune reaction to engineered implants should occur.

The quality of cells obtained from urine is similar to that of the biopsy-derived cells described above. When differentiated, USC express all proteins characteristic of the various bladder cell lineages. Karyotype analysis has demonstrated that these cells are genetically stable. Importantly, there is a major cost advantage to using USC – it costs about $50 to obtain cells from urine, versus about $5,000 to isolate cells from a biopsy procedure[6]. About 1.4 $x10^9$ urothelial and smooth muscle cells (SMC) are required for bladder tissue regeneration[63]. We estimate that 3-4 urine samples (about 40-45 USC/600 ml urine) expanded for 4-5 weeks would yield a sufficient quantity of low passage, healthy cells for clinical tissue engineering applications. This time frame is comparable to that required for expansion from a tissue biopsy (7-8 weeks)[64]. USC and the cells obtained through urological tissue biopsies come from the same urinary tract systems and have similar biological features. Therefore, collecting cells from urine could be an attractive alternative to the standard urological tissue biopsies currently used in cell therapy and tissue engineering.

3. Stem/progenitor cells derived from non-urological tissues

Despite the convenience of using differentiated cells in tissue engineering applications, these cells have several shortcomings. These cells have a limited ability to grow in culture and they tend to dedifferentiate *in vitro*, which may lead to insufficient numbers of cells. In addition, autologous bladder cells cannot be taken from patients with urinary tract malignancies. One solution to these problems is to prepare engineered tissues using stem cells from various sources. These types of stem/progenitor cells from non-urological tissue have been studied as cell sources for bladder regeneration and cell therapy for stress urinary incontinence.

Mesenchymal stem cells

Mesenchymal stem cells (MSC), isolated from bone marrow, skeletal muscle, and adipose tissue, possess the capacity to differentiate into cells of connective tissue lineages, including muscle. Isolation and characterization of MSC, and control of their myogenic differentiation derived from both pre-clinical and clinical studies have attracted attention to their potential use in urological regenerative medicine and tissue engineering.

Currently, the most effectively characterized types of multipotent stem cells are from bone marrow. Bone marrow stem cells (BMSC) have been shown to differentiate into specialized cells, including hepatocytes[65-67], neural cells[68-71] and mainly mesodermal derivatives such as bone, cartilage, cardiac muscles, skeletal muscle, and fat. If BMSC are placed on a proper

bio-degradable scaffold and implanted, they can act as anti-fibrotic, angiogenic, anti-apoptotic, and mitotic agents. Recently, BMSC were evaluated as an alternative cell type for use in replacement of bladder SMC when native bladder muscle tissue is unavailable. The potential of BMSC to differentiate into cells with bladder SMC characteristics was assessed i*n vitro*[72] and in different animal models [73-78]. Kanematsu et al[75] showed that *in vitro,* both supernatants from cultured rat bladder cells (conditioned media) or media containing TGF-β and VEGF induced bone marrow cells to adopt a SMC phenotype. Recently, we have investigated the impacts of soluble growth factors, bladder extracellular matrix (ECM), and 3D dynamic culture on cell proliferation and differentiation of human BMSC into smooth muscle cells (SMC)[72]. Myogenic growth factors (PDGF-BB and TGF-β1) alone, or combined either with bladder ECM or dynamic cultures, induced BMSC to express smooth muscle specific genes and proteins. Either ECM or the dynamic culture alone promoted cell proliferation but did not induce myogenic differentiation of BMSC. A highly porous nanofibrous poly-L-lactic acid (PLLA) scaffold provided a 3D structure for maximizing the cell-matrix penetration, maintained myogenic differentiation of the induced BMSC, and promoted tissue remolding with rich capillary formation *in vivo*. This study demonstrates that myogenic-differentiated BMSC seeded on a nanofibrous PLLA scaffold can be used for cell-based tissue engineering for bladder cancer patients requiring cystoplasty.

In order to test this *in vivo,* bone marrow cells expressing green fluorescent protein were transplanted into lethally irradiated rats. Eight weeks following transplantation, bladder domes were replaced with acellular matrix grafts. Two weeks after the graft procedure, GFP expression in the matrices indicated that the transplanted marrow cells had repopulated the graft. By 12 weeks, these cells reconstituted the smooth muscle layer, with native SMC also infiltrating the graft. In another rat study[74], rapid regeneration of bladder SMC and urothelium occurred on BMSC seeded collagen matrices, whereas fibrotic changes were observed in the non-seeded matrix group 3 months after bladder augmentation. In a large animal study[73], BMSC proliferated at the same rate as primary cultured bladder SMC *in vitro*, and they had a similar histological appearance and contractile phenotype as primary cultured bladder SMC. BMSC had a significant contractile response to calcium-ionophore *in vitro,* and this response was similar to that seen in bladder SMC but markedly different from fibroblasts. Immunohistochemical staining and Western blotting indicated that BMSC expressed α -smooth muscle actin, but did not express desmin or myosin. *In vivo,* small intestinal submucosa (SIS) grafts seeded with BMSC developed solid smooth-muscle bundle formations throughout the grafts, as did bladder cell-seeded SIS grafts. However, bladder tissue regeneration did not occur in animals that received cell-free scaffolding. These results indicate that BMSC may provide an alternative cell source for bladder tissue engineering. This is relevant for patients with bladder malignancies who require bladder augmentation or replacement but do not have enough normal, non-malignant bladder cells to use in tissue engineering applications.

Other MSC such as skeletal muscle-derived progenitor cells [79-88] [89-92] and adipose stem cells[80, 90, 91, 93-98] have been investigated as potential candidates for cell-based tissue engineering and injection therapy stress urinary incontinence (SUI), and these studies are further described in Section 5.

Induced pluripotent stem cells

iPS cells are a type of pluripotent stem cell that is artificially derived from a patient's own somatic cells (a non-pluripotent cell) by inducing a "forced" expression of certain genes. iPS

cells were first produced in 2006 from mouse cells and then in 2007 from human cells. iPS cells are typically derived by transfecting stem cell-associated genes into non-pluripotent cells, such as adult fibroblasts. Transfection is typically achieved through viral vectors, such as retroviruses. Transfected genes include the master transcriptional regulators Oct-3/4 (Pouf51) and Sox2, although it is suggested that other genes may enhance the efficiency of induction. After 3–4 weeks, small numbers of transfected cells begin to become morphologically and biochemically similar to pluripotent stem cells, and these cells are typically isolated through morphological selection or through a reporter gene and/or antibiotic selection. This has been cited as an important advancement in stem cell research, as it may allow researchers to obtain pluripotent stem cells, which are important in research and potentially have therapeutic uses in urology, without the controversial use of embryos.

iPS cells are believed to be similar to natural pluripotent stem cells, such as embryonic stem (ES) cells in many respects, including expression of certain stem cell genes and proteins, chromatin methylation patterns, doubling time, embryoid body formation, teratoma formation, viable chimera formation, potency and differentiability, but the full extent of their relation to natural pluripotent stem cells is still being assessed.

However, depending on the methods used, reprogramming of adult cells to obtain iPS cells may pose significant risks that currently limit the use of this technique in human therapy. For example, if viruses are used to genetically alter the cells, expression of oncogenes may potentially be triggered. In February 2008, a report published in the journal *Cell* announced the discovery of a technique that removed the need for oncogenes such as c-myc in induction of pluripotency, thereby increasing the potential use of iPS cells in human diseases. Even more recently, in April 2009, Sheng Ding in La Jolla, California, showed that the generation of iPS cells was possible without any genetic alteration of the adult cell [99]. Repeated treatment of the cells with certain proteins channeled into the cells via poly-arginine anchors was sufficient to induce pluripotency. The cells generated by this process are known as protein-induced pluripotent stem cells (piPS cells).

Human Amniotic Fluid Stem Cells

Human amniotic fluid cells are commonly used clinically as a diagnostic tool for the prenatal diagnosis of fetal genetic anomalies. Recently, increasing evidence demonstrated that fetal-derived stem cells can be isolated from amniotic fluid. These cells represent a novel class of pluripotent stem cells with intermediate characteristics between embryonic and adult stem cells, as they are capable of giving rise to lineages representative of all three germ layers but do not form teratomas when implanted *in vivo*[100]. These features, in addition to the absence of ethical concerns about their use, indicate that amniotic fluid stem (AFS) cells might be a promising cell source for tissue engineering and stem cell therapy. Perin et al [101-103] have recently reported that AFS cells may be useful for kidney regeneration. In a series of studies, this group demonstrated that these pluripotent cells are able to differentiate into *de novo* kidney structures during organogenesis *in vitro.* Human male amniotic fluid cells were isolated between 12 and 18 weeks of gestation. AFS cells were isolated from these cultures and labeled with green fluorescent protein and Lac-Z protein. Labeled human AFS cells were then microinjected into murine embryonic kidneys (12.5–18 days gestation) and these were maintained in a co-culture system for 10 days. Histological analysis revealed that human AFS cells were able to contribute to the development of elemental kidney structures including renal vesicles, and C- and S-shaped bodies.

Expression of the early kidney markers zona occludens-1, glial-derived neurotrophic factor and claudin were confirmed by RT-PCR. Therefore, it is possible that amniotic fluid stem cells represent a potential cell source for future renal cell therapies.

4. Approaches for inducing myogenic differentiation of stem cells

When stem cells are used as a cell source for urological tissue engineering and regeneration, they can be used three ways: 1) stem cells can be induced to differentiate into the target cells/tissue–like cells *in vitro* before cell implantation; 2) stem cells are implanted directly into the tissues where repair is needed, and the surrounding cell- and tissue-based signals induce the stem cells to differentiate into the specific cells required for regeneration, and 3) a cell-free scaffold is implanted and recruits the host's own stem/progenitor cells, which then differentiate into the proper cell type required for tissue repair.

Current research tends to focus largely on the first strategy described above to allow control over the signals that the cells receive so that differentiation into the tissue type required progresses without problems. For this strategy, it is necessary to mimic the physiological conditions that guide stem cells to differentiate into the desired target cells before implantation. For example, several factors have been shown to enhance autologous adult stem cell differentiation into functional SMC, including:

i. Growth factors, such as vessel endothelial growth factor (VEGF), platelet-derived growth factor (PDGF-BB), transforming growth factor-β (TGF-β) and insulin-like growth factor (IGF)[104],
ii. Components of the extracellular matrix (ECM). Cellular interactions with the ECM play an important role in cell adhesion, growth, migration, apoptosis and differentiation [105]. It consists of compounds such as collagen, laminin [106], and fibronectin. Collagen IV can promote embryonic stem cells to differentiate into stem cell antigen-1-positive (Sca-1$^+$) progenitor cells and SMC[107]. However, ECM can promote adult cell proliferation or improve the yield of SMC derived from adult MSC, but ECM alone cannot induce adult MSC to differentiate into SMC[108, 109]. Additionally, culture on a three-dimensional ECM scaffold in a dynamic culture system can improve cell proliferation, maintain cell phenotypes and lead to a more homogenous distribution of cells on the scaffold [110, 111] when compared to 2-D static culture.
iii. *In vitro* co-culture of fully-differentiated SMC and stem cells appears to improve stem cell differentiation into muscle cells, most likely because the SMC secrete specific factors into the culture medium. Baskin et al demonstrated that mature urothelium can induce urological embryonic tissue or stem cells to differentiate into smooth muscle cells *in vivo* through epithelial-stromal cell interaction or cell-cell interaction. In contrast, the embryonic tissue failed to differentiate into SMC when urothelium was not present[112]. Because of this, conditioned medium is commonly used for stem cell differentiation. Conditioned medium is essentially culture medium that is partially used by cells, and it is enriched with cell-derived material including small amounts of growth factors.
iv. The application of cyclic mechanical strain to cell cultures has been demonstrated to increase the expression of smooth muscle cell markers in stem cells[113]. Periodic stretching occurs *in vivo* as a part of the natural function of hollow organs; for example, as the bladder fills and empties. Differentiated SMC easily lose their contractile function in static culture once the cells leave the body, but the use of mechanical strain in culture

can prevent this spontaneous loss of phenotype *in vitro* and maintain SMC functional characteristics[114].

5. Autologous stem cells for endoscopic therapies

Another exciting area of clinical urologic investigation is the use of various autologous cells to treat vesicoureteral reflux (VUR) and SUI. An increasing number of clinical trials using tissue engineering approaches have been reported (Table 1). All of these clinical applications of urological tissue engineering are based on a series of successful animal experiments[115, 116, 117].

Cell types	Require Tissue biopsy	Highly expandable	Potential applications	Clinical trail
Skeleton muscle derived progenitor cells	Yes		Cell therapy for VRU and urinary incontinent, Bladder regeneration	Yes
Bone marrow stem cells	Yes	Depend on age	Tissue engineered bladder; Cell therapy for VRU; urinary incontinent	Yes
Adipose stem cells	Yes		Tissue engineered bladder;	Yes
Urine progenitor cells	No	no depend on age	Tissue engineered urological organs	No

Table 1. Potential use of autologous stem/progenitor cells for urological tissue engineering and cell therapy

Endoscopic therapy offers a simple method for definitive treatment in SUI and VUR. Two types of injectable substances have been investigated. First, natural and synthetic biomaterials that serve as bulking agents, such as silicone, fibrin, bioglass, polyvinyl alcohol foam, alginate gel, a small-intestinal submucosal suspension and Defulx have been used[118]. Currently, injectable therapy based on bulking agents is used for only about two-thirds of patients with SUI, with even lower cure rates. Potential problems of these injectable substances include decreasing volume of the injectable substance over time *in vivo*, the need for multiple injections to obtain and maintain optimal efficacy, potential antigenicity of the injectable and related allergic reactions, migration of the injected material, and urethral pain both at the time of injection and afterward. The ideal bulking agent should remain efficacious over time and have few side effects, but so far, none of the substances in use have met these criteria for success and the search for a superior injectable therapy for SUI continues.

Cell-based therapy is a promising alternative in urological procedures for VUR and USI. Autologous cells that can be used for this purpose include chondrocytes[115, 119], adipose-

derived stem cells (ASC) [120-122], BMSC [121, 123], and skeletal muscle derived progenitor cells [85, 124-127]. In 1994, Atala et al proposed the use of injectable autologous chondrocytes to correct VUR via endoscopy [12, 128]. Using a minipig model, they noted that autologous chondrocytes injected around the ureter to stop reflux did not migrate and the cartilage bead produced by this technique maintained its volume with time. Reflux was corrected in all animals treated endoscopically with autologous chondrocytes. After these successful animal experiments, several clinical trials have begun[115, 119]. One study [129] was conducted in a total of 29 children (46 ureters) with grades II to IV reflux. Chondrocytes were harvested from a biopsy of each patient's ear cartilage and were grown in culture for 6 weeks. Patients then returned to the clinic for transurethral injection of chondrocytes into the bladder trigone to correct reflux. Ultrasound was performed 1 month after this procedure and radionuclide cystography was done 3 months postoperatively to confirm reflux resolution. When reflux persisted, repeat treatment with stored chondrocytes was offered. In this study, a single chondrocyte injection corrected reflux in 26 of the 46 ureters (57%), while secondary injection was successful in 12 of 19 (63%). Overall, reflux was corrected in 38 of the 46 ureters (83%) and in 24 of the 29 patients (83%). There were no significant complications, and transurethral injection of autologous chondrocytes to correct VUR in children appears to be an effective and safe technique. The only limitation of this therapy is the high cost.

The use of myocyte- and stem cell-based injection therapy has also been tested in VUR cases. Primary VUR is a congenital anomaly of the ureter-vesical junction that creates a deficiency of the longitudinal muscle of the intravesical ureter. This leads to an inadequate valvular mechanism and allows urine to flow backward from the bladder to the kidney. Thus, myocyte-based therapies are attractive options for the recovery of this muscle defect at the ureteral orifice.

Autologous progenitor cell-based therapy has also made significant progress in treatment of SUI. This cell therapy could soon become a standard procedure. The objective of this therapy is to improve or cure the sphincter dysfunction via periurethral endoscopic injection. Currently, myoblasts obtained from skeletal muscle biopsies and adipose-derived cells are the most commonly used cells for therapy for SUI. Recently, autologous myoblasts and fibroblasts have been evaluated as a potential injectable therapy for SUI. One group[130-133] has studied a combination therapy consisting of autologous myoblasts injected into the rhabdosphincter and fibroblasts injected into the urethral submucosa. A year follow-up study of 123 women was performed from 2004 to 2005. A cure rate of about 79% with improvements in quality-of-life scores, rhabdosphincter contractility, and urethral closure pressures has been achieved. All patients were continent 1 year after receiving this therapy and maintained their good outcome at further follow-up visits. Ultrasound images before treatment clearly revealed poor periurethral integrity of the sphincteric mechanism; postinjection images revealed a completely normal-appearing urethra.

Rodriguez *et al* recently reported that adipose-derived stem cells (ASC) have the potential to differentiate into functional SMC[89] . ASC expressed a series of contractile proteins, including α-SM actin, desmin, myosin heavy chain, calponin, caldesomon, smoothlin, and SM22 following aspiration from fat tissue and culture in SM differentiation medium. One important advantage of using ASC is that adipose tissue can be harvested in large quantities with minimal morbidity. Autologous fat tissues were used for cell injection therapy for vesicoureteral reflux in a clinical study [134]. Two out of 11 patients had a reduction in grade of reflux, including one ureter that ceased refluxing altogether[135]. One recent study[136]

showed ASC could correct neurogenic erectile dysfunction in rats as effectively as bone marrow stem cells did. More research is underway to determine whether ASC can differentiate into Leydig, Sertoli and male germ cells. The eventual goal of the research is to use ASC to treat male infertility and testosterone deficiency.

6. Conclusions

Current advances in urological tissue engineering and stem cell-based therapy demonstrate that bladder and urethral tissues can be regenerated using autologous cells seeded onto biodegradable scaffolds. VUR and SUI can be corrected with injections of autologous stem cells contained in a hydrogel. However, many issues must be elucidated before these techniques can become widely used in the clinic. For example, the role of donor cells in tissue regeneration remains unclear, and it is not known whether the seeded stem cells proliferate and populate scaffold materials themselves, or if they stimulate to the activation, migration, proliferation, and differentiation of the local progenitor cells to complete the tissue regeneration. Additionally, an approach to promote angiogenesis and to facilitate innervation with a functional network of regenerated nerves will greatly improve tissue regeneration strategies to create a *de novo* urological organ. (6,015 words)

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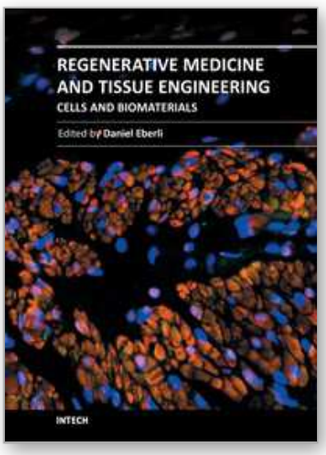

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Tissue Engineering may offer new treatment alternatives for organ replacement or repair deteriorated organs. Among the clinical applications of Tissue Engineering are the production of artificial skin for burn patients, tissue engineered trachea, cartilage for knee-replacement procedures, urinary bladder replacement, urethra substitutes and cellular therapies for the treatment of urinary incontinence. The Tissue Engineering approach has major advantages over traditional organ transplantation and circumvents the problem of organ shortage. Tissues reconstructed from readily available biopsy material induce only minimal or no immunogenicity when reimplanted in the patient. This book is aimed at anyone interested in the application of Tissue Engineering in different organ systems. It offers insights into a wide variety of strategies applying the principles of Tissue Engineering to tissue and organ regeneration.

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