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Review

# Urothelial Differentiation of Mesenchymal Stem Cells for Potential Application in Urinary Tract Tissue Regeneration

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**Abstract:** Urothelial cells (UCs), with functional tight junctions, act as a barrier of urinary tract, autologous UCs provide a cell source for tissue engineering in urology, but healthy UCs might be not available under the circumstances of malignancy, infection and stones in urinary tract system. Mesenchymal stem cells (MSCs) from various tissues, such as bone marrow, adipose tissue, amniotic fluid and voided urine are investigated on urothelial differentiation for urinary tract regeneration. In this brief review we discussed urothelial differentiation of MSCs, especially urine derived stem cells, which provide potential application in urinary tract epithelial reconstruction.

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**Keywords:** Mesenchymal stem cell, Stem cell, Tissue Regeneration, Urothelial cells, Urinary tract

## 1. Introduction

The urothelium, the epithelium lining the surface of the urinary tract including renalpelvis, ureterandbladder, is a unique cell type with high plasticity and multiple functions [1-3]. When the urothelium is compromised during injury or inflammation, it can result in the passage of toxic substances into the underlying tissue (neural/muscle layers) resulting in urgency, frequency and pain during voiding [4]. The urothelium responds to the pressure in bladder, stretch during the filling phase and contract during the empting phase of micturition reflex. What's more, urothelium plays an important role in the injuries and infections, including control of permeability, immune response, and cell-cell communications [5].

Bladder is frequently suffered from infections, lithiasis, dysfunction, cancer formation, or congenital malformations (e.g. bladder exstrophy). Although bladder is located within the bony pelvis, it still occurred with blunt and penetrating trauma. Some of these cases caused urothelial cells lose or suffered with abnormal structure and function, caused reduced contractility and compliance, form heavy scar tissue, and significantly reduce bladder volume (end-stage bladder disease), which should be corrected or regenerated with tissue engineering [6, 7]. For bladder tissue engineering, urothelial cells (UCs) are needed for creating bladder mucosa, smooth muscle cells (SMCs) for building up bladder wall, and endothelial cells (ECs) for forming blood vessels, and urothelial cells are vital important for bladder regeneration. However, generating urothelial cells is still a challenge.

Urothelial cells are endoderm cell lineage, it is hard to recover large area of defect although they reconstruct local injury rapidly. Mesenchymal stem cells (MSCs) are multiple somatic stem cells, wildly used in regenerative medicine, while it is difficult to induce MSCs to give rise to urothelial cells with low efficiency.

We recently demonstrated that a subpopulation of cells isolated from urine possess biological characteristics similar to mesenchymal stem cells (MSCs), and termed these cells "urine-derived stem cells" or USCs. USCs consistently expressed MSC/pericyte markers, but not hematopoietic stem cell markers (except for MHC-1), endothelial cell markers (CD31). Compared to other MSCs, USCs have several advantages: i) they

can be collected using a simple, safe, low-cost and non-invasive procedure; ii) they display telomerase activity so that they are able to generate more cells; iii) they differentiate into SMCs, UCs and ECs with high efficiency; and iV) they are originated from urinary system, supposed to be more suitable for urothelial differentiation and applied in bladder regeneration.

In this review, we evaluate MSCs application in urinary tract tissue regeneration through the following standard :i) urothelial gene and protein, to evaluate the urothelial differentiation potential and efficiency; ii) multilayer formation; iii) barrier function evaluation through tight junction genes and proteins expression, ultrastructure and barrier function assay.

## 2. Urothelial Cell

The urothelium is composed of at least 3 layers of cells, the superficial, intermediate and basal cells, physically forms the first line of urinary defense. The superficial umbrella cells, which are interconnected by tight junctions, play an important role in maintaining the barrier, and exhibit a number of properties including specialized membrane lipids, asymmetric unit membrane particles and a plasmalemma with stiff plaques [1-3]. Apical urothelial cells function as a barrier against most substances found in urine thus protecting the underlying tissues [1, 3]. The basal cell layer is the location of urothelial stem / progenitor cells. Although urothelial cells can proliferate and reconstruct the injured bladder epithelium rapidly physically, while at the circumstance of large patch lose or dysfunction, the residual urothelium cannot meet the need of mucosa recovery. The urothelium cannot proliferate and expand efficiently in vitro, it exhibits very limited proliferation and passage ability. So urothelium differentiated from stem cells are desired for urinary regenerative medicine.

## 3. Urothelial Differentiation of Mesenchymal Stem Cell

As a multipotential stem cell lineage, MSCs could differentiate into the desired type of tissue to be used successfully in therapies. Via trans-differentiation, MSCs can give rise to all three types of cells in the bladder, including urothelial cells[10]. In addition, MSCs possess paracrine effects with angiogenic, anti-apoptosis, anti-fibrosis, anti-inflammatory properties. Hypoxic stress increases generation of several of these cytokines and growth factors. Thus, MSCs can recruit resident stem cells participating in tissue repair. Furthermore, MSCs purportedly exhibit low immunogenicity, allowing allogeneic applications.

MSCs have several advantages for tissue repair: i) other than ESCs and iPSCs, they do not induce teratoma or malignant tumors; ii) they can generate a large amount of cells within 4 weeks; iii) they are highly efficient in giving rise to functional bladder cells, such as UCs; iv) they secrete paracrine factors that allow stem cells to be tolerated by the host's immune system; and v) their use avoids general ethical concerns that accompany use of other types of stem cells.

### *3.1 Bone marrow derived mesenchymal stem cells (BMSCs), adipose tissue derived stem cells (ASCs), and amniotic fluid-derived stem cells (AFSCs)*

BMSCs, ASCs, AFSCs and even endometrial stem cells are investigated the urothelial differentiation ability, they have some limitations (Table 1): low differentiation capacity (5%~50%) of UCs (endodermal lineage); short lifespan in vitro (<10 passages in BMSCs); and they require invasive collection procedures [10-17]. One of the most likely reasons for this is that true stem cells in bone marrow stromal cells are very rare, depending on donor age (1/104 cells in newborns, but 1/106 in older individuals). Furthermore, it is very difficult to isolate stem cells from the large amount of somatic cells.

Thus, the ideal stem cell sources for urothelial differentiation in bladder repair would i) be able to differentiate into functional UCs with tight junction formation; ii) allow collection via a non-invasive, simple, safe, and low-cost method; iii) have universal availability; and iv) generate tissue-specific or organ-specific stem cells from the urinary tract system. Currently, it is unknown whether such a 'perfect' stem cell exists. We do know, however, that certain cell types are more favorable than others.

### *3.2 Urine derived stem cell*

#### *3.2.1 General features of USCs*

We recently found that a subpopulation of cells isolated from urine, USCs, possess biological characteristics similar to MSCs, i.e. clonogenicity, cell growth patterns, expansion capacity [18-20], cell surface

**Table 1. Comparison of USCs and other MSCs in urothelial differentiation potential**

| | Positive control | Negative control | Urothelial differentiation of MSCs | | |
|---|---|---|---|---|---|
| | Urothelial cells | Non-induced USC | USCs | BMSCs[10] Or ASCs[12] | AFSCs[13] |
| Induction approach | | | CM-UC; or EGF (30ng/ml) | CM-UC; or EGF (30ng/ml) | bladder cancer-derived conditioned medium |
| Morphology | Cobblestone | Rice-grain | Cobblestone | Spindle | - |
| Urothelial gene expression (UropalinkIa/III, cytokeratin) | 4+ | -~1+ | 3+~4+ | 1+~2+ | 1+~2+ |
| Urothelial protein expression (UropalinkIa/III, cytokeratin) | 4+ | -~1+ | 3+~4+ | 1+~2+ | 1+~2+ |
| *-via Western-blot* | 4+ | -~1+ | 3+~4+ | 1+~2+ | 1+~2+ |
| *-via immunofluorescence staining* | 4+ | -~1+ | 3+~4+ | 1+~2+ | 1+~2+ |
| Barrier function | 4+ | - | 3+~4+ | NA | - |
| Tight junction gene expression | 4+ | - | 3+~4+ | NA | NA |
| Tight junction protein expression | | | | | |
| *-via Western-Blot* | 4+ | - | 3+~4+ | NA | NA |
| *-via immunofluorescence staining* | 4+ | - | 3+~4+ | NA | |
| SEM | + | - | + | - | - |
| Multilayers Urothelial formation on biomaterials in vitro | 4+ | - | 3+~4+ | + | NA |
| Urothelial protein expression when differentiated USCs seeded on biomaterials in vivo | 4+ | -~1+ | 3+~4+ | +~3+ | NA |
| Paracrine effects | 1+~4+ | 3+ | 4+ | 4+ | 3+~4+ |
| Induction ratio | | | 60%~70% | 5%~50% | NA |
| Expand ability | + | 4+ | 4+ | 2+~3+ | 2+~3+ |
| Easy get | + | 4+ | | | |
| Available for autologous trans-plantation | + | 4+ | 3+~4+ | 3+~4+ | - |

Abbreviation: UCs,urothelial cells; USCs, urine derived stem cells; BMSCs, bone marrow derived mesenchymal stem cells; ASCs, adipose derived stem cells; AFSCs, amniotic fluid-derived stem cells; CM-UC, conditioned medium from cultured urothelial cells

marker expression profiles [20], multipotent differentiation capacity[19-22], [23-25], pro-angiogenic paracrine effects [26, 27], immunomodulatory properties[28] and easily-induced iPS cells[29]. USCs consistently expressed MSCs markers and some key cell surface markers, but not hematopoietic stem cell markers (except forMHC-1), endothelial cell markers (CD31), or human leukocyte antigen(locus) DR (HLA-DR). Our data demonstrated that USCs are capable of osteogenic, chondrogenic, adipogenic, myogenic, neurogenic and endothelial differentiation. After being induced in the appropriate condition in vitro, each type of differentiated USCs expressed specific markers at the gene, protein, cellular levels. Following implantation in vivo, induced USCs can form functional bone, cartilage, fat, muscle, endothelium, and urothelium tissue [30]. Compared to other MSCs, USCs have several advantages: i) they can be collected using a simple, safe, low-cost and non-invasive procedure; ii) they display telomerase activity so that they are able to generate more cells; and iii) they differentiate into UCs, SMCs and ECs with high efficiency.

USCs can be obtained from voided urine and can generate a large number of cells from a single clone [23]. These cells possess highly proliferative capacity because they maintain higher telomerase activity and longer telomere length compared to BMSCs. Up to 75% of USCs collected from middle-aged individuals expressed telomerase activity (USCs-TA$^+$) and retained long telomere length[31], but USCs-TA$^+$ decline to 50-60% of the USCs in people older than 50. USCs-TA$^+$ can be maintained for up to 20 passage with 67 population doublings (PD), indicating that a single USC can generate up to 267 cells within 14 weeks. In contrast, USCs-TA- grow only for 8-10 passages with 34 PD. Importantly, either USCs-TA$^+$ or USCs-TA$^-$ display normal karyotypes in culture medium even after several passages. They do not form teratoma 3 months after renal subcapsular cell implantation [31]. We can now obtain 100-140 USC clones/24 hr urine from each individual [18]. About 1.4 x 109 cells are needed for potential use in bladder reconstruction with cell-seeded technology [32]. Thus, two urine samples containing 20-30 USC clones in 400 ml can provide ample cells (1.5 x109USCs at p4) with 4-5 weeks to be used in cell-based therapy for bladder repair.

*3.2.2 Urothelial differentiation of USCs*

Using the same inductive condition as in the BMSC study [10], we found that 60%-70% of USCs differentiated into cells expressing uro-epithelial cell-specific genes (uroplakin-Ia/III) and protein markers, and had urothelial barrier function and tight junction ultrastructures, formed multilayer cells. Urothelial differentiated USCs also expressed the genes and proteins for ZO-1, E-cadherin, and cingulin (associated with tight junctions) in a dose- and time-dependent manner. The barrier function of induced USCs reaches the mature function of UCs isolated from bladder tissue 14 days after induction, significantly higher than for non-induced USCs, indicating that USCs possessed stem cell plasticity.

We recently demonstrated that USCs can impart profound immunomodulatory effects and the cells, inhibit proliferation of peripheral blood mononuclear cells (PBMNC, T and B cells), and secrete interleukin (IL)-6 and IL-8[28]. PBMNCs proliferated when mixed with other cells due to immune stimulation. The PBMNC concentration in USC wells were much less than that in BMSC culture wells. These results indicating that less immunological reaction will be stimulated when USCs differentiated UCs are used in bladder regeneration compared with BMSCs.

## 4. Debated hypotheses

Several assumptions are still controversial in urothelial differentiation of MSCs: i) Trans-differentiation and paracrine effects are both critical in regeneration of various tissues. Although most studies monitored the survival rate of implanted cells, cytokines and growth factors secreted from stem cells might play an important role in urothelial differentiation in bladder repair. ii) Should undifferentiated or differentiated MSCs be used in urothelial cells needed in bladder repair? Undifferentiated stem cells can secrete more paracrine factors than differentiated stem cells, but differentiated cells might possess more potential to replace dysfunctional somatic cells. Therefore, a 1:1 ratio of undifferentiated and differentiated cells might be optimal for bladder regeneration.

## 5. Future Directions

UCs seeded on the luminal side of scaffold are often lost during surgery procedures, washed out via the urine, mechanically ejected via the urethral catheter. In addition, successfully retained cells start to die within the first week, most probably due to ischemia, inflammation, or apoptosis due to detachment from the

extracellular matrix. Therefore, it is extremely important to increase viability of implanted stem cells early after cell transplantation. Several methods might help reach this goal: i) using biomaterials with porous micro-structure that might protect cell retention within the scaffold; ii) keeping the cell-seeding scaffold construct wet in the culture media, and avoid drying it out during surgery.

## 6. Take Home Messages

MSCs possess an excellent feasibility and safety profile for bladder tissue regeneration. Pre-clinical outcomes have been generally positive in restoring bladder contractility and volume the partial (40%) cystoplasty model. Autologous MSCs derived from patients would be a potential cell source for the bladder repair. MSCs appear safeto use for urological tissue repair with no evidence of increased tumorigenesis after implantation. Urinary-derived stem cells possess MSC features including self-renewal, multi-differentiation potential, and paracrine effects. As a novel cell source, USCs can be obtained via a non-invasive, simple, safe and low-cost approach, are highly expandable, express telomerase activity but do not induce teratoma, and can give rise to urothelial cells efficiently, with tight junction formation, is the optimal stem cell source for urothelial differentiation in urinary tract tissue regeneration so far.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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