Diabetic men often have Erectile Dysfunction (ED) (arrange from 20% to 75%), and it often occurs 10-15 years earlier, and is more severe, than in non-diabetic men [1-3]. Diabetes-related ED involves impairments in endothelial cells that affect blood vessel, smooth muscle, and nerve function [4]. The endothelium’s shift to a vasoconstrictor, pro-thrombotic and pro-inflammatory state is a major factor in development of diabetic ED. The pathogenesis of both endothelial Dysfunction and Diabetic ED is linked through decreased expression and activation of Endothelial Nitric Oxide Synthesis (eNOS), and the subsequent blunted physiological actions of NO occurring with diabetes. The mechanisms involved include impaired endothelial eNOS function in the cavern sum, smooth muscle cell dysfunction, and neurodegradation from increased generation of oxidative stress and overproduction of free radicals and Reactive Oxygen Species (ROS) [5,6,7]. Endothelial dysfunction is a major contributor to the high incidence of ED in patients with diabetes, particularly in the early stages [8-11]. It is critical to address erectile function before diabetic ED moves to an advanced stage. Oral medications are an important first-line treatment for ED, but only 50-60% of patients see improvements from these drugs [12] because diabetic ED decreases NO production. These results have motivated investigators to seek novel treatment approaches, one of which is stem cell therapy.

Mesenchymal Stem Cells (MSCs) can differentiate into various cell types, including endothelial, smooth muscle, Schwann cells, and neurons. In addition, MSCs can secrete paracrine factors and cytokines that enhance cell survival and angiogenesis, and promote anti-apoptotic, pro-angiogenic, anti-inflammatory, and anti-fibrotic effects [13]. Intracavernous transplantation of Bone Marrow-Derived Stromal Cells (BMSCs) [14-16] or adipose stem cells (ASCs) [5,17] increased the number of eNOS-positive endothelial cells and smooth muscle content [5,15] and Numbers of Neuronal Nitric Oxide Synthesis (nNOS)-positive nerve fibers [14,17,18] in the corpus cavern sum in a rodent model. However, to obtain these stem cells, bone marrow or fat aspiration is usually required, with possible complications. MSCs used in most studies of diabetic ED were isolated from healthy donors [5,17,19-23] and not from patients’ own tissues. For eventual clinical use, autologous stem cells would be optimal because they would not cause immune rejection or other adverse events associated with allergenic or exogenous sources. Thus, autologous stem cells obtained from a non-invasive, safe, reproducible, and low-cost approach would be highly desirable.

We discovered a subpopulation of cells isolated from urine that possess biological characteristics similar to adult stem cells, i.e. clonogenicity, cell growth patterns, expansion capacity [24-26] cell surface marker expression profiles, multipotent differentiation capacity [24-35] including endothelial and smooth muscle differentiation [24-26] pro-angiogenicparacrine effects [25,26,28,29] immunomodulatory properties [32] and easily-induced Induced Pluripotent Stem (iPS) cells [36,37]. We have termed these cells “Urine-Derived Stem Cells” (USCs). USCs are not MSCs and they most likely originate from the kidney [26]. Our recent study showed that USCs give rise to functional endothelial cells [31,38] and myocytes [28,30,34,39] besides estrogenic, chondrogenic and adipogenic cell lineages in vitro and in vivo [24-26]. Like bone marrow stem cells, USCs also secrete antigenic growth factors and cytokines. In addition, USCs inhibit peripheral blood mononuclear cell (T and B cell) proliferation and secrete the immunoregulatory cytokines interleukin (IL)-6 and IL-832. Our procedures with USCs have been successfully repeated by independent investigators in other institutes [27,37,40-49].

Obtained from healthy donors’ voided urine, USCs can generate a large number of cells from a single clone [30, 35]. Importantly, 57-75% of the USCs collected from middle-aged individuals
expressed telomerase activity (USCs-TA+) and retained long telomere length50. USCs-TA+ possessed higher proliferative capacities and were maintained for up to 67 Population Doublings (PD), indicating that a single USC can generate up to 267 cells within 14 weeks. We can consistently obtain 100-140 USC clones/24 hr urine from each individual [24]. About 1 x106 cells are needed for use in diabetic ED therapy in a rodent model [5,51,52]. Thus, one 200 ml urine sample can provide ample cells for the purposes of cell implantation. Although USCs express certain embryonic stem cell markers and displayed telomerase activity, these cells displayed genetic stability after serial passages of culture and did not form any tumor clones in vitro [25,35]. No cancer cells appeared 3 months after USC implantation, either subcutaneously or under renal capsules [50].

The studies form others and us have demonstrated that ASCs or ASCs expressing VEGF significantly improved the erectile function in streptozotocin (STZ)-induced ED rat model [15,17]. Our recent studies demonstrated that use of implanted USCs obtained from healthy human donors or USCs expressing FGFR2 displayed significantly higher Intracavernous Pressure/Mean Arterial Pressure (ICP/MAP) ratios 28 days after Intracavernous injection in vivo [53]. In addition, USCs or USCs expressing FGFR2 were associated with significantly increased expression of endothelial markers (CD31, VEGF and eNOS), smooth muscle markers (desmin and smoothelin) compared to normal saline injection in the diabetic ED rat model [53]. Although few cells were detected within the implanted sites, histological and western blot analysis demonstrated an increased expression of endothelial and smooth muscle markers within the cavernous tissue following USC or USC-FGFR2 injection. This study indicated that the paracrine effect of USCs or USCs-FGFR2 induced improvement of erectile function in type 2 diabetic rats by recruiting resident cells and increasing the endothelial expression and contents of smooth muscle [53].

In addition, our most recent study showed that human USCs significantly improved renal function in a rat model of chronic renal insufficiency induced by gentamicin combined with renal ischemic insult, with a 50% decline in serum creatinine 2 weeks post-cell injection (5x106cells/kidney) maintained over 9 weeks, compared to controls. The implanted USCs were detected around the glomerulus and interstitial area. Numbers of macrophages and amount of collagen deposit significantly decreased in USC-treated nude rats. Furthermore, either local administration via per urethral injection of USCs or systemic administration via Intraperitoneal Injection (i.p.) significantly enhanced the sphincter function by increasing leak point pressure, and restored histologic features by protection against urethral sphincter injuries in a rat model one week after vaginal distention injury (unpublished data). These in vivo studies indicate that USCs promote tissue regeneration, reduce inflammation and improve urogenic function via paracrine effects and cell differentiation.

Taken together, analogous USCs provide an alternative cell source for cell therapy in treatment of andro-urological diseases including diabetes-related ED. The mechanism of USC therapy is involved with cell differentiation and paracrine effect to induce the endogenous regeneration potential.

References


