Aims: In this study, we investigated the differences between mesenchymal stem cells (MSCs), isolated from umbilical cord blood (UCB-MSCs) and Wharton’s jelly (WJ-MSCs) as sources of diabetes mellitus cell therapy. Methods: After isolation, both cell types were induced to differentiate into insulin producing cells, then the differentiated cells were assessed genetically and functionally. UCB-MSCs and WJ-MSCs were transplanted in the tail veins of streptozotocin-induced diabetic rats. Blood glucose levels were monitored post-transplantation. Results & conclusion: Wharton’s jelly was more homogeneous, can better differentiate into insulin producing cells in vitro and better control hyperglycemia in diabetic rats in vivo, as compared with UCB. These results indicate that WJ-MSCs represent a potential source of cells in the field of diabetes mellitus cell therapy.

Keywords: cell banking • cell therapy • diabetes mellitus • mesenchymal stem cells • umbilical cord blood • Wharton’s jelly

Type 1 diabetes mellitus (T1DM) is an autoimmune disorder in which the body’s immune system attacks and destroys pancreatic β cells. While insulin replacement represents the current therapy for T1DM, it hardly controls diabetes, nor does it necessarily prevent the possibility of the disease’s devastating long-term complications, which can seriously affect every body organ [1]. Pancreatic or islet transplantation can provide exogenous insulin independence, but is limited by several factors including its intrinsic complications and organ donor’s scarcity [2].

The major goal of future diabetes therapy is to promote β-cell regeneration, which could be accomplished by β-cell self-replication or differentiation from progenitor cells with the use of stem cell therapy to overcome autoimmunity and to improve endogenous insulin secretion [2]. Today, significant effort is being made to find alternative means to treat diabetes through stem cell therapy. Several reports have been published concerning the differentiation of many kinds of stem cells into insulin producing cells (IPCs), including embryonic stem cells [3], pancreatic stem cells [4] and mesenchymal stem cells (MSCs) derived from either bone marrow [5] or umbilical cord (UC) [6].

MSCs are uniquely capable of crossing germinative layers borders (i.e., are able to differentiate toward different embryonic lineages including ectoderm-, mesoderm- and endoderm-derived cytotypes) and are viewed as promising cells for regenerative medicine approaches in several diseases [7]. MSCs are obtainable in high numbers via ex vivo culture [5]. In addition, other reports evidenced that MSCs possess immune-modulatory activities which should result in a reduction of the immunogenicity of transplanted cells, thus limiting rejection [8]. However, caution is still needed to ensure safe and durable effects of these MSCs in vivo.

Interestingly, UC has been proved to be a good source of MSCs either from umbilical cord blood (UCB) or from Wharton’s jelly (WJ) – the connective tissue surrounding the umbilical vessels [9,10]. These MSCs offer several advantages over other types of stem cells [7]. For example, UC-MSCs are
easily isolated compared with embryonic stem cells (ESCs). Moreover, MSCs possess immune-modulatory properties which prevent rejections to occur even after xenotransplantation of postdifferentiated MSCs without immunosuppression [11]. Furthermore, they are obtainable in high numbers and can be differentiated to IPCs. Thus, UC-MSCs represent a promising therapeutic target and a potential source for cell replacement therapy for diabetes mellitus (DM) [12].

MSCs isolated from different tissues using different methods of administration have been used for treating DM in animal models [13,14]. Although these MSCs exhibit several common characteristics, some important differences according to their origin have been shown with regard to their morphology, colony formation abilities, differentiation capacities and therapeutic effects [15,16].

Accordingly, we sought to isolate, propagate and characterize MSCs from two sources of UC, namely UCB-MSCs and WJ-MSCs as noninvasive and readily available sources of stem cells. Furthermore, we compared these two promising types of cells for their pancreatic differentiation potential in vitro and their ability to control hyperglycemia in streptozotocin (STZ)-induced diabetic rats in vivo. Our results concluded WJ-MSCs as more potential candidate for diabetes cell therapy than UC-MSCs and should be strongly recommended for stem cell banking and DM cell therapy.

Materials & methods
Isolation & culture of UCB-MSCs & WJ-MSCs

The umbilical cords were obtained from Obstetrics/Gynecology Department, Ain Shams after obtaining a signed informed consent from the mother. Fresh human UCB and UC were maintained on ice and processed within 1–4 h post delivery.

UCB mononuclear cells (UCB-MNCs) were isolated as described previously [17]. Briefly, UCB was collected on sterile 3.2% citrate solution as an anticoagulant. UCB was diluted with phosphate buffer saline (PBS) in the ratio 1:1, then 30 ml of the diluted blood was layered carefully on 10 ml of Ficoll-hypaque (Histopaque 1.077 g/ml density, Sigma-Aldrich, MO, USA) in 50 ml Falcon tubes and then centrifuged at 2000 rpm for 30 min at room temperature. Mononuclear cells were collected from plasma/ficoll interphase and washed twice with PBS. Contaminating erythrocytes were lysed with ammonium chloride lysis solution, and then washed with PBS. Cell density was adjusted to 1–2 × 10⁶/ml and plated in 500 μl of FACS buffer and analyzed by CYTOMICS FC 500 Flow Cytometer (Beckman Coulter, Marseille, France). Mouse isotype IgG1 FITC and PE antibodies were employed as controls. Then, the cells were washed and suspended in 500 μl of FACS buffer and analyzed by CYTOMICS FC 500 Flow Cytometer (Beckman Coulter, FL, USA) using CXP Software version 2.2.

All immunophenotyping assays were done in Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt.

Adipogenic & osteogenic differentiation

We performed adipogenic and osteogenic differentiation as examples of specific mesenchymal lineage
using human MSC functional identification kit (R&D systems, Inc., MN, USA). This kit contains specially formulated adipogenic media supplement containing hydrocortisone, isobutyryl methyl xanthine and indomethacin in 95% ethanol which can be used to effectively differentiate MSCs into adipogenic lineage. Briefly, cells were cultured in a 24-well tissue culture plate at a density of 3.7 × 10^4/well in α-MEM Basal Medium supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin and 2 mM l-glutamine (Lonza) and incubated at 37°C in a humidified atmosphere of 5% CO2. When the cells reached 100% confluency, the medium was replaced with adipogenic differentiation medium to induce adipogenesis. After 5–7 days, lipid vacuoles started to appear in the induced cells. The detection of the resultant differentiated cells was done using Oil red staining (Sigma-Aldrich), as described previously [19]. As for osteogenic lineage, 4.2 × 10^3 cells were seeded in 24-well plate. When these cells reached 50–70% confluency, the medium was replaced with osteogenic medium supplemented with the kit and kept for 21 days with every 3–4 days change of the medium. Differentiation was confirmed by Alizarin red-S staining (Sigma-Aldrich) for the calcium rich extracellular matrix [19].

Pancreatic lineage differentiation
After 2–4 passages, both UCB-MSCs and WJ-MSCs were induced to differentiate into IPCs using a three step protocol as described previously [20]. Briefly, P2 ~ P4 cells were induced by 5% FBS high-glucose DMEM (4.5 g/l glucose) for 14 days (step I), then 10 mmol/l nicotinamide (Sigma-Aldrich) was added for 7 days (step II), and then finally 10 nmol/l exendin-4 (Sigma-Aldrich) was added for another 7 days (step III).

RNA extraction & Real-time PCR analysis
Both control undifferentiated UCB-MSCs and WJ-MSCs, together with differentiated IPCs (at steps II and III) were collected. RNA was isolated using Trizol Reagent (Life Technologies, CA, USA) according to the manufacturer’s instructions. Briefly, 3 × 10^6 cells were treated by 1 ml Trizol followed by extraction using chloroform and isopropanol. RNA was precipitated by 80% ethanol. cDNA was prepared by Verso™ cDNA synthesis kit (Thermo Scientific, MA, USA) using 0.5 μg RNA. Each quantitative RT-PCR (qRT-PCR) was done using 4 ng cDNA using SYBR Green Master Mix (Applied Biosystems, CA, USA). GAPDH was used as internal control. ΔΔ Ct method was used to calculate relative expression levels. The mRNA expression of various markers was done by qRT-PCR. Forward and reverse primers for target genes are given in (Table 1). All qRT-PCR analyses were done on Step-One plus qRT-PCR (Applied Biosystems).

Functional assessment of differentiated cells by glucose challenge test for insulin release
The maturity of differentiated IPCs was assessed by its ability to secrete insulin in response to high glucose [21]. Briefly, the differentiated cells were washed twice with PBS then incubated for 1 h in Kreb’s Ringer bicarbonate (KRB) buffer supplemented with 5.5 mM glucose at 37°C, 5% CO2. Afterward, cells were incubated with either 5.5 mM, 16.7 mM or 25.5 mM glucose in KRB buffer in the same conditions for 2 h, and then the supernatant was collected and frozen at -80°C till time of assay. Insulin release was detected by AccuBind® insulin enzyme-linked immunosorbent assay (Monobind Inc., CA, USA) according to the manufacturer’s instructions.

Transplantation of UCB-MSCs & WJ-MSCs into streptozotocin-induced diabetic rats
A total of 40 male Sprague–Dawley rats of 4–5 weeks old and 100–150 gm weight were purchased from the Animal Center of the Nile Company for Pharmaceuticals and Chemical Industries, Cairo, Egypt. Animals were housed in normal cages at controlled temperature (24°C) with a 12:12 h light:dark cycle and had free access to water and chow diet over a 5-week

| Table 1. Forward and reverse primer sequences used for quantitative real-time PCR. |
|---------------------------------|-------------------------------|-------------------------------|
| **Gene** | **Forward primer** | **Reverse primer** |
| GAPDH | GCCAAAAGGGTACATCATCTC | TGAGCTTTTCCAGGATACCA |
| Nestin | CGTGGCAACAGGAGTGGGA | AGGCAGGAGGACATGTGAG |
| Pdx-1 | GAAGCAGGAGGACAAAG | CTGGTCAAAGTCAAGACAG |
| MafA | CTGGTCCAGCTGACGTC | CAGAGCTGCGGGAGGAG |
| Ngn-3 | TCAAAGTGGACCGGTGAGAC | AGCTGACACTCGTGCTC |
| Nkx2.2 | TCTAGCAGAGACGCGCAAC | TTGCTCCGGCCGCCTA |
| Isl-1 | ATTTCCCTATGTGTTGGTTCG | CGTTCCTGCTGAAGCCGATG |
adaptation period in animal house facility till reaching the ideal weight of STZ injection (200–300 g). Experimental diabetes was induced in these 40 rats (9–10 weeks age, weighing 200–300 gm) by single intraperitoneal injection of 50 mg/kg BW STZ (Sigma-Aldrich) in 0.1 M citrate buffer [22]. Each rat was injected in the right lower part of its abdomen within 30 min of the STZ solution preparation to avoid the degradation of STZ. One week after STZ injection, the rats were food deprived overnight the day prior to blood glucose measurement. The terminal part of rat tail was rubbed by xylene wetted cotton ball to show the tail veins. After xylene dried out, the tail vein was punctured by needle to obtain blood drop. The blood drop was applied on the strip of glucometer (Bionime, Shanghai, China) to measure fasting blood glucose (FBG). Rats having blood glucose level of 200 mg/dl or greater were considered to be diabetic. These recorded FBG levels were used in the subsequent experiment as day 0 (D0) FBG before cells transplantation. In the same day, bodyweights (BW) of the diabetic rats were measured and recorded as D0 BW. Special care was taken to provide enough water and food for diabetic rats.

Of these 40 STZ diabetic-induced rats, 18 rats were selected randomly for stem cells transplantation. These 18 rats were divided randomly into three groups; control group (seven rats), UCB-MSCs group (five rats) and WJ-MSCs group (six rats). Each group was placed in separate cages. On day of injection, each rat received 2 × 10^6 freshly trypsinized P3 either UCB-MSCs or WJ-MSCs, each according to its corresponding group, suspended in 300 μl plain LG-DMEM through tail vein. Again, the tail was rubbed by xylene and after dried out, cells were transplanted into tail veins using 1 ml 100 unit 27 gauge insulin syringe (BD) on day 7 after STZ induction of diabetes. Same volume of plain LG-DMEM was injected into control group diabetic rats. FBG and BW were monitored every 10 days up to 8 weeks post-transplantation as discussed earlier in this section.

Two months post-transplantation, 2–3 rats of each group were sacrificed by cervical dislocation and dissected for organs collections. Autopsy samples were taken from the pancreas, livers, kidneys and spleens of rats in different groups, along with normal rats. Hematoxylin and eosin (H&E) stained slides were prepared as described before [23]. Briefly, samples were washed with water followed by gradient alcohol for dehydration. Paraffin tissue blocks of these specimens were prepared and sectioned at 4 μm thickness by sledge microtome. Then, obtained tissue sections were collected on glass slides, deparaffinized, stained by H&E stain and examined under light microscope.

**Immunohistochemistry**

Immunostaining for insulin was done as previously described [24]. Briefly, paraffin sections were deparaffinized and rehydrated by serial changes of xylene, ethanol and distilled water. Antigen retrieval was done by citrate buffer in steamer at 95°C for 30 min. After blocking, slides were incubated with anti-human Insulin (A0564, DAKO, CA, USA) for 1 h followed by incubation with secondary antibody MACH-2 Horse Radish Peroxidase polymer (Biocare Medical, CA, USA). Color was developed using DAB chromogen kit (DAKO, CA, USA) as per manufacturer’s instructions and visualized under light microscope.

**Statistical analyses**

Data are presented as mean ± standard error of mean. Comparisons between the groups were conducted using one-way analysis of variance and Dunnett’s post hoc test. These statistical analyses were done using windows-based SPSS statistical package (SPSS version 17.0; SPSS, IL, USA). A p-value of less than 0.05 was considered significant.

**Results**

**Both UCB & WJ are sources of mesenchymal stem cells**

As for UCB, isolated cells started to adhere to plastic surface in 5–7 days following density gradient isolation. In the beginning, these cells formed an adherent heterogeneous cell population consisting of round and spindle shaped cells (UCB-MNCs; Figure 1A). Initially, these cells proliferate slowly and reach confluency within 3–4 weeks. Upon subculture, this heterogeneous cell population changed to a homogeneous one with flat fibroblast-like shaped cells (UCB-MSCs; Figure 1B).

On the other hand, adherent cells with fibroblast-like morphology could be observed as early as 10–14 days post-plating of the explants of WJ. As shown in Figure 1C & D, these cells were almost homogeneous resembling MSCs morphology, as further proved by immunophenotyping. These cells were designated as WJ-MSCs.

**Immunophenotyping of cells isolated from UCB & WJ**

Isolated fibroblast-like cells from both UCB and WJ were characterized by flow cytometry for MSCs and hematopoietic-specific cluster of differentiation (CDs) markers. As shown in Figure 2A & B, immunophenotyping revealed that there exists difference in the expression of these markers in both types of cells. Both types were almost negative for CD14 (monocytes), CD34 (hematopoietic stem cells) and CD45 (leukocyte-specific antigen) with more percentage of
Figure 1. Phase contrast images of umbilical cord blood and Wharton’s jelly-isolated cells. (A) Freshly isolated UCB-mononuclear cells showing heterogeneous population, (B) UCB-mesenchymal stem cells after first passage (P1) showing homogeneous fibroblast-like cells and (C) freshly isolated WJ-mesenchymal stem cells showing homogeneous fibroblast-like cells which continues for P1 as shown in (D). Magnification: 10×; Scale bar: 100 μm. UCB: Umbilical cord blood; WJ: Wharton’s jelly.

cells expressing these markers in the UCB population (CD14 UCB: 4.8% vs WJ: 1.5%; CD34 UCB: 8.6% vs WJ: 2.6%; CD45 UCB: 13.3% vs WJ: 6.3%). On the other hand, both cell types were positive for MSCs markers CD44, 73, 90 and 105. Interestingly, there were difference in expression intensities of these markers between both types of cells (CD44 UCB: 85.1% vs WJ: 83.6%; CD73 UCB: 71.9% vs WJ: 83.4%; CD90 UCB: 74.1% vs WJ 90%; CD105 UCB: 77.9% vs WJ: 82.2%). These results indicate more homogeneous mesenchymal phenotypic population of WJ-MSCs as compared with those of UCB-MSCs.

Adipogenic & osteogenic differentiation of UCB-MSCs & WJ-MSCs

As a functional assay to confirm MSC identity in isolated cells, we examined the differentiation potential of both of these cell populations. Although isolation yields and immunophenotyping profile differ between these two populations, their mesenchymal lineage differentiation capacity either to adipogenic or osteogenic differentiation remained conserved. As Figure 2C–H shows, both UCB-MSCs (C–E) and WJ-MSCs (F–H) exhibited both adipogenic differentiation potential; detected by oil red staining of lipid droplets in control with undifferentiated cells and osteogenic differentiation potential of the isolated WJ-MSCs; detected by alizarin-red-S staining for calcium rich extracellular matrix as compared with control undifferentiated cells.

In vitro differentiation of UCB-MSCs & WJ-MSCs into IPCs: morphological changes & gene expression analysis

Following exposure to differentiation protocols, both cell types start to lose their fibroblast-like shape and tend to aggregate by the end of nicotinamide (NA) stage. This goes on with exendin-4 stage. Furthermore, cells start to detach and grow as suspension in the culture medium (Figure 3A–D). However, control cells keep their MSCs like morphology throughout the differentiation period.

In order to evaluate the potential of these types of MSCs to differentiate into IPCs, we examined the gene expression of β cells-related genes by qRT-PCR. We examined Nestin; a stem cell marker; Pdx-1, MafA, Ngn-3, Nkx2.2 and Isl-1 as β-cell differentiation markers. As shown in Figure 4A, Nestin transcript level was decreased in both differentiated UCB-MSCs and WJ-MSCs. It is obvious here that level of Nestin transcript in undifferentiated UCB-MSCs was higher as compared with WJ-MSCs.

This decrease in Nestin levels upon differentiation was associated with concomitant increase in expression of β-cells genes in both types of cells including Pdx-1, MafA, Ngn-3, Nkx2.2 and Isl-1 either in NA stage or at final differentiation stage as shown in Figure 4B & C. Interestingly, all these genes showed a considerable higher fold increase in final differentiated cells when compared with undifferentiated cells in WJ-MSCs than in UCB-MSCs (Pdx-1, UCB: 2.5-fold the expression level in undifferentiated control, WJ-MSCs: 14.7-fold; MafA, UCB: 2.5-fold, WJ: 3.1-fold; Ngn-3, UCBP: 4.9-fold, WJ-MSCs: 6.4-fold; Nkx2.2, UCB: 0.27-fold, WJ-MSCs: 7.6-fold; Isl-1, UCB: 1.88-fold, WJ-MSCs: 2.8-fold). These findings indicate that although both cells could potentially differentiate down the pancreatic lineage, WJ-MSCs showed increased potentiality toward differentiation into IPCs.

Comparison of glucose-stimulated insulin secretion of IPCs generated from both UCB-MSCs & WJ-MSCs

One important characteristic of β cells is its secretion of insulin in response to glucose [21]. In order to examine the response of differentiated IPCs to glucose stimulation, we incubated differentiated cells with KRB buffer containing a low (5.5 mM) or two high (16.7 and 25 mM) glucose concentrations. ELISA showed a modest secretion of insulin in response to glucose for IPCs isolated from UCB-MSC (LG: 5.62 ± 0.063 μU/ml; high glucose (HG) 16.7mM: 5.77 ± 0.176 μU/ml; HG 25 mM: 5.88 ± 0.290 μU/ml) or WJ-MSCs (LG: 5.21 ± 0.11 μU/ml; HG 16.7mM: 5.93 ± 0.11 μU/ml;
Figure 2. Characterization of mesenchymal stem cells from umbilical cord blood and Wharton’s jelly by immunophenotyping and mesenchymal lineage differentiation (see facing page). Immunophenotyping of cells isolated from (A) UCB and (B) WJ. Cells were labeled with FITC- or PE-conjugated antibodies and examined by flow cytometry. The immunophenotypical profile of both UCB-MSCs and WJ-MSCs showed low expression of CD14, CD34 and CD45 in both types of cells with more cells expressing these markers among UCB. On the other hand, both were positive for CD44, 73, 90 and 105 with more expression intensity for WJ-MSC than UCB-MSC. (C–H) Adipogenic and osteogenic differentiation of UCB and WJ-MSCs. (C) Uninduced UCB-MSCs as control for lineage differentiation, (D) induced UCB-MSCs showing red staining of oil droplets using oil red, characteristic for successful adipogenic differentiation, (E) induced UCB-MSCs showing positive alizarin red-S staining for calcium rich extracellular matrix, indicating successful osteogenic differentiation, (F) uninduced control WJ-MSCs, (G) induced WJ-MSCs showing red staining of oil droplets using oil red, characteristic for successful adipogenic differentiation, (H) induced WJ-MSCs showing positive alizarin red-S staining for calcium rich extracellular matrix, indicating successful osteogenic differentiation.

FITC: Fluorescein isothiocyanate; MSC: Mesenchymal stem cell; PE: Phycoerythrin; UCB: Umbilical cord blood; WJ: Wharton’s jelly.

HG 25 mM: 5.79 ± 0.15 μU/ml. However, as shown in Figure 4D, the increase in insulin secretion was slightly higher but significant in WJ-MSCs as compared with UCB-MSCs. Yet, there was no significant variation of insulin secretions between the two high-glucose concentrations.

Transplantation of UCB-MSCs & WJ-MSCs into STZ-induced rats

The diabetes treatment potentials of both types of cells were tested in vivo in STZ-induced diabetic rat model. Either UCB-MSCs or WJ-MSCs suspended in plain LG-DMEM media were transplanted into STZ-induced diabetic rats together with another group injected plain LG-DMEM media serving as control group. Fasting blood glucose and BW were recorded every 10 days for 2 months. As shown in Figure 5A, rats transplanted with UCB-MSCs showed decreased FBG starting from day 40 (D40) post-transplantation (UCB-MSCs FBG at D40: 401.2 ± 24.7 mg/dl compared with control D40: 521.0 ± 16.11 mg/dl) and reached its lowest level at D50 (UCB-MSCs FBG at D50: 282.6 ± 29.1 mg/dl compared with control D50: 540 ± 29.80 mg/dl) Unfortunately, these cells could not manage to keep the decreased FBG, which started to elevate to approach control levels at D60 post-transplantation.

On the other hand, the WJ-MSCs transplanted rats showed decreased FBG levels starting at D10 (WJ-MSCs FBG at D10: 209.0 ± 18.5 mg/dl compared with control D10: 378.5 ± 12.85 mg/dl) with sustained decreased FBG levels till D50. However, FBG started to elevate again after D50 where it approached FBG in control group (WJ-MSCs FBG at D50: 439.7 ± 56.2 mg/dl compared with control D50: 540.0 ± 29.80 mg/dl). Then decreased again significantly from control group at D60 (WJ-MSCs FBG at D60: 356.2 ± 45.2 mg/dl compared with control D60: 576.6 ± 8.9 mg/dl). Comparison of the FBG levels between UCB-MSCs group and WJ-MSCs at different time points of the study is shown in Supplementary Figure 1.

Figure 3. Phase contrast images of differentiated umbilical cord blood-mesenchymal stem cells and Wharton’s jelly-mesenchymal stem cells into insulin producing cells. Upon differentiation, both induced UCB-MSCs (B) and induced WJ-MSCs (D) aggregate to form clusters in contrast to control UCB-MSCs (A) and WJ-MSCs, (C) which retain fibroblast-like morphology. Magnification: 10×; Scale bar: 100 μm.

UCB-MSC: Umbilical cord blood-mesenchymal stem cell; WJ-MSC: Wharton’s jelly-mesenchymal stem cell.
and stained them with H&E for routine examination. In addition, we performed immunostaining for human insulin in these pancreata. As shown in Figure 6A & B, the H&E staining of pancreas of the normal control group, showed no histopathological alterations. Meanwhile, it showed some immunostaining for insulin may be due to cross-reactivity of the antibody with other mammalian insulins. As for the rats injected with STZ, expectedly, this group showed dilated pancreatic ducts associated with edema in the periductal tissue. Importantly, the islets of Langerhans were atrophied and showed very little insulin immunostaining (Figure 6C & D).

As for the rats transplanted with MSCs, histopathological examination showed that there was a dramatic improvement in the pancreata of the rats treated with either UCB-MSCs or WJ-MSCs. In contrast to dilated edematous ducts and atrophied islets shown in STZ group, both UCB-MSCs and WJ-MSCs totally restored the normal histological structures of acini and more importantly, islets of Langerhans (Figure 6E & G).

Figure 4. Gene expression of insulin producing cells generated from umbilical cord blood-mesenchymal stem cells and Wharton’s jelly-mesenchymal stem cells. (A) Real-time PCR revealed relative decrease in Nestin levels in both UCB and WJ-differentiated cells with increased expression of Nestin in noninduced UCB-MSC as compared with WJ-MSC. Fold increase of mRNA relative expression level of β-cells genes in (B) UCB-MSC and (C) WJ-MSC showed increased expression of Pdx-1, MafA, Nkx2.2, Ngn-3 and Isl-1 in both types of cells with more consistent increase associated with insulin producing cells generated from WJ-MSCs. (D) In vitro GSIS assay of differentiated UCB-MSCs and WJ-MSCs. Insulin release in response to a low (5.5 mM) and two high (16.7 and 25 mM) glucose concentrations of differentiated cell cluster was measured after 2 h incubation.

*Mean is significantly different from control mean at p < 0.05.

GSIS: Glucose-stimulated insulin secretion; MSC: Mesenchymal stem cell; NA: Nicotinamide; UCB: Umbilical cord blood; WJ: Wharton’s jelly.
Figure 5. Fasting blood glucose and bodyweight loss in streptozotocin-induced diabetic rats treated with mesenchymal stem cells. (A) Fasting blood glucose and (B) bodyweight loss in rats transplanted with UCB-MSCs and WJ-MSCs as compared with control group injected plain Low glucose-Dulbecco’s Modified Eagle Medium media. Transplantation of UCB-MSCs and WJ-MSCs decreased FBG and bodyweight loss as compared with control group.

*Mean is significantly different from control mean at p < 0.05.

#Mean is significantly different from UCB-MSCs mean at p < 0.05.

FBG: Fasting blood glucose; UCB-MSC: Umbilical cord blood-mesenchymal stem cell; WJ-MSC: Wharton’s jelly-mesenchymal stem cell.

histopathological structures of all organs. On the other hand, expectedly, rats injected with STZ (Supplementary Figure 2D–F) showed dilated congested veins associated with inflammatory cells infiltration in between the hepatocytes as well as in the portal area with edema in the later. The kidneys showed degeneration and desquamation in the lining epithelium. In spleen, severe congestion was noticed in the red pulps, while the white one showed lymphoid depletion.

In the rats transplanted with MSCs, Supplementary Figure 2G–I show that the histopathological changes induced by STZ in either liver, including edema and congestion in portal veins, or kidney, including vacuolization in the lining endothelium of the glomerular tufts, were not affected by either transplantation of UCB-MSCs or WJ-MSCs. On the other hand, spleen autopsies showed no histopathological alteration in rats treated with UCB-MSCs (Supplementary Figure 2I). However, spleens of rats transplanted with WJ-MSCs showed lymphoid depletion in the white pulps (Supplementary Figure 2L).

Discussion

In this study, we compared two important banking types of MSCs isolated from UC, namely UCB-MSCs and WJ-MSCs regarding their potentialities toward the generation of IPCs in vitro and the control of hyperglycemia in STZ-induced diabetic rats as potential source of DM cell therapy. Results of this study showed that both cell types, UCB-MSCs and WJ-MSCs exhibit typical MSCs characteristics; however, WJ-MSCs were superior to UCB-MSCs in ease of isolation and propagation. In addition, although both cell types failed to attain fully differentiated IPCs in vitro or completely ameliorate hyperglycemia in vivo, WJ-MSCs exhibited better differentiation potential to IPCs and better sustained control of hyperglycemia in STZ diabetic rats. These results strongly indicate WJ-MSCs can be considered as more potential candidate for DM cell therapy as compared with UCB-MSCs, and should be strongly recommended for stem cell banking.

Stem cells regeneration offers an attractive insulin replacement therapy for those with insulin-dependent DM. Stem cells from pancreas [25], bone marrow [5], UCB [26] have been previously used in research for regeneration therapies for DM. In this context, UC is considered a readily available source of MSCs. In addition, both UCB-MSCs and WJ-MSCs are obtained from tissues that are discarded after delivery, and this nullifies any ethical concerns that might be raised about the use of these cells. Moreover, MSCs have several immune-modulatory properties which enhance their potential for the use in cell therapy of DM [7].

Actually, the International Society for Cellular Therapy suggested three criteria for describing MSCs [27]. The first one is plastic adherence. We succeeded to isolate plastic adherent MSCs-like cells from both types of cells. However, the elapsed time between samples collection and appearance of these MSCs was variable. While it took only about 2 weeks to get the fibroblast-like cells to appear from WJ samples, UCB started with more heterogeneous population, took more than a month to isolate MSC-like cells. Moreover, WJ-MSCs could be expanded more efficiently than UCB-MSCs.

These results supported previous studies on WJ-MSCs in comparison with other MSCs including peripheral blood [28] and bone marrow [29]. Taking into consideration that the preference of using cells in
of cells, both types of cells showed similar multilineage differentiation evidenced by ability of both types to exhibit adipogenic and osteogenic differentiation.

Previous studies showed plausible pancreatic differentiation from embryonic stem cells [30] and bone marrow MSCs [31]. Moreover, several reports have shown the potential of generating IPCs from UCB-MSCs [6,17] and WJ-MSCs [32] or even from other sources of MSCs like adipose tissue [33]. In our study, both types of cells were induced into IPCs after they were cultured in pancreatic differentiation medium containing successive addition of NA and exendin-4 to high-glucose DMEM (4.5 g/l). Basically, differentiation was assessed both genetically and functionally. Interestingly, the more fold increase in β-cells differentiation markers showed by WJ-MSCs as compared with undifferentiated cells upon differentiation into IPCs, which reached almost double that of UCB-MSCs for every gene, suggests a better potentiality of these cells in differentiation into IPCs rather than IPCs generated from UCB-MSCs. Basically, this added more weight to the suitability of WJ-MSCs in cell therapy for diabetes especially in future clinical settings.

However, the formation of mature glucose responsive cells from these sources is a major challenge in the field of diabetes cellular therapy. Several studies have demonstrated that the small amount of insulin secreted by these cells in vitro may not be very useful clinically [34]. In addition, several reports assume that stem cells from different origins generate immature IPCs [7].

These previous observations were confirmed in the glucose-stimulated insulin secretion experiment. Although differentiated cells showed increased secretion of insulin upon challenge with high glucose concentration, the response did not significantly change with higher glucose concentrations. This might indicate that resultant cells, although express several β-cell development genes but still did not manage to attain fully functional mature β cells.

Recently, a special attention has been drawn toward the role played by Nestin in differentiation of stem cells into islet-like cells. Nestin is an intermediate filament protein transiently expressed during early development in neuronal cells as well as in embryonic and adult cells [35]. In pancreas, it is considered a marker of pancreatic stem cells and islets progenitor cells [36]. Actually, La Rocca and colleagues, 2009, were among the first groups to show that WJ-MSCs express neuroectodermal marker Nestin [37]. Interestingly, another study showed that MSCs from UC which constitutively express Nestin were ideal candidate source for islet neogenesis and diabetes cell replacement therapy [38]. Moreover, another study demonstrated that knockdown of...
Nestin by gene silencing in either embryonic stem cells or pancreatic ductal stem cells leads to poor pancreatic differentiation and decreased insulin secretion [39].

However, in our study, Nestin transcripts levels decreased upon differentiation in both UCB-MSCs and WJ-MSCs. Interestingly, the transcript level of Nestin in UCB-MSCs was higher than that of WJ-MSCs, although WJ-MSCs showed more expression of β-cell genes upon differentiation, that is, showed higher potential toward generating IPCs. In other words, higher Nestin expression was associated with cells with lower differentiation potential, which was somewhat different from outcomes from previously mentioned studies. One explanation is that these studies were using either embryonic stem cells or pancreatic stem cells. However, it seems that the role of Nestin in pancreatic differentiation of MSCs is quite different and might need further elucidation.

One prominent feature that was shown in both UCB-MSCs and WJ-MSCs is the efficient expression of MafA in both types of cells upon differentiation to IPCs. In addition, the expression of MafA was more prominent in IPCs generated from WJ-MSCs in comparison with UCB-MSCs. MafA is a transcription factor responsible for insulin synthesis. This indicates that the potential of WJ-MSCs to express insulin gene and secrete insulin in response to glucose will be superior to that of UCB-MSCs. This was confirmed in glucose-stimulated insulin secretion assay, where WJ-MSCs showed slightly higher sensitivity to glucose challenge than UCB-MSCs. Again, we can conclude from these results that WJ-MSCs may represent as a better potential candidate for diabetes cellular therapy than UCB-MSCs.

A number of previous studies and clinical trials have revealed that MSCs are capable of reducing glucose levels in animals or subjects with Type 1 DM [40,41]. Therefore, we considered the transplantation of these two types of MSCs into STZ-induced diabetic rats. Interestingly, the better potential demonstrated by WJ-MSCs in vitro, evidenced by higher expression of pancreatic β-cell genes at end stage of differentiation, was completely reflected in vivo when these cells were transplanted into STZ-induced diabetic rats. Basically, WJ-MSCs managed to decrease FBG in an earlier and more sustainable fashion as compared with UCB-MSCs. Moreover, the bodyweight loss usually associated with diabetes was greatly improved by both UCB-MSCs and WJ-MSCs transplantation.

These results of our in vivo experiments were in accordance with several previous reports. Phuc and coworkers, 2011, showed that IPCs generated from cryopreserved UCB cells were able to just decrease elevation of glucose in STZ-induced diabetic rats as compared with control [17]. In another study, transplantation of IPCs derived from WJ-MSCs into portal vein could significantly decrease blood glucose level in transplanted animals and human C-peptide was detected in livers of transplanted animals [32]. In addition, a recent paper by Si and colleagues, 2012, showed that MSCs treatment ameliorated hyperglycemia in rats with Type 2 DM not only by β-cell restoration but also by improving insulin sensitivity [42]. Interestingly, another recent study by Liu and colleagues, 2014, showed that treatment of Type 2 DM patients with WJ-MSCs could improve metabolic control and β-cell function [43].

It is worth noting here that we used undifferentiated cells in transplantation because undifferentiated cells will provide advantageous use in clinical setting including ease of isolation and expansion with lack of need for manipulation before transplantation. This was the rational after our preference of undifferentiated cells over differentiated ones.

Based on the ability of these MSCs to ameliorate hyperglycemia in diabetic rats, we collected autopsy samples of liver, kidney, spleen and pancreas, which are considered plausible target organs of STZ. Interestingly, H&E staining and insulin immunostaining showed that both UCB-MSCs and WJ-MSCs transplantation ameliorated, at least partially, the damage induced by STZ in the pancreas and produced a similar morphology to normal islets. Based on current knowledge, it was considered that the underlying mechanism of the therapeutic effect of MSCs on hyperglycemia might involve islet regeneration, through direct differentiation into functionally competent β cells [11,44]. Interestingly, a recent study showed that MSCs may promote β-cell regeneration, not through differentiation into β cells, but through cytokine-dependent recruitment of macrophages, which in turn, activate β-cell regeneration [45]. Further studies are warranted to fully elucidate the mechanism by which MSCs can promote β-cell regeneration. Moreover, our results also were consistent with the notion that transplanted MSCs possess tissue repair and cytoprotective properties possibly due to their preferential homing properties to acute-injured tissue [46]. However, in our experiments, MSCs induced tissue repair only in pancreatic lesions with minimal effects on other damaged tissues. Nevertheless, MSCs did not exhibit this effect on any other STZ-induced damaged organ.

It is noteworthy here that some controversial studies have suggested that the limited number of MSC-derived functional β cells in vivo and the small amount of insulin produced by these cells seemed
to be inadequate to maintain euglycemia [8,46]. This important concern was completely reflected in our hands both in vitro and in vivo. Lack of glucose responsiveness in differentiated IPCs in vitro together with the inability of MSCs to ameliorate hyperglycemia in diabetic rats, might indicate that although MSCs of either sources exhibit the ability to differentiate into IPCs in vitro and restore damaged pancreas in vivo, still these differentiated cells did not attain fully functional β-cells phenotype. This might represent a direct challenge in stem cell therapy of diabetes. Future directions and studies should be focusing on how to improve such differentiation if MSCs are to be added to our battle against diabetes.

Actually failure of both cell types to attain fully differentiated IPCs represents a major limitation of this study. Another limitations may include the cross-reactivity of human insulin antibody with rat insulin. This cross-reactivity prevented us from determining if the beneficial effects showed by the MSCs in this study are due to generation human pancreatic cells in vivo or regeneration of rat pancreatic β-cells. However, this mechanism warrants further studies for elucidation. Also, we determined the gene expression levels of β-cell markers by detecting the transcript level by qRT-PCR. However, this can be validated by detecting protein levels in further studies.

Another interesting finding revealed in our histopathological study was that WJ-MSCs sustained a lymphoid depletion in spleen indicating an immune-modulatory effect of these MSCs. This was not the case for UCB-MSCs. Several reports have shown the immune-modulatory effect of MSCs and how this might present MSCs as double-edged sword in diabetes treatment [8,47]. Recent studies showed that MSCs could be ‘per se’ used in treatment of Type 1 DM [40,48]. This additional benefit gives more weight to WJ-MSCs as a potential candidate in treatment of DM.

Briefly, our findings in this study clearly demonstrate that umbilical cord is a rich source of MSCs, either from UCB or WJ. Interestingly, both represent important cell banking sources for further use by individual during later life. Importantly, our results demonstrate WJ as more rich and readily available source of MSCs when compared with CB. Moreover, WJ-MSCs showed higher differentiation potential toward IPCs in vitro and more promising FBG lowering effect in vivo in STZ-induced diabetic rats, yet, both types of cells failed to attain fully functional IPCs either in vitro or in vivo. These findings shed lights on both importance and relative feasibility demonstrated by WJ-MSCs over UCB-MSCs as a potential source of cell therapy for diabetes.

**Conclusion**

Our results show that human UC represent a rich source of MSCs from either CB or WJ. MSCs isolated from both origins were able to differentiate to pancreatic lineage cells in vitro and can alleviate hyperglycemia in vivo. Thus, these cells represent a readily available, promising stem cell source for β-cell regeneration. Basically, WJ-MSCs offer advantageous source of cells for diabetes cell therapy when compared with UCB-MSCs. In light of these findings, one would recommend more consideration of WJ-MSCs as source of cell banking for further use later in life. Although the abundance of literature suggests that generation of IPCs from stem cells is feasible, many considerations such as source of cells, induction protocols and mechanisms of differentiation, should be further explored before the application of these cells to clinical treatment of diabetes.

**Future perspective**

This study is comparing two types of MSCs isolated from UC, namely UCB-MSCs and WJ-MSCs. MSCs are considered strong candidates for use in regenerative medicine and tissue repair. This is attributed in part to their good culture characteristics, which fulfil the need of huge number of cells in clinical applications. In addition, these cells exhibit multipotency, which allow them to generate different organs even from different embryonic lineages. Moreover, these cells may modulate immune responses in host, which may find its way to both allogenic and heterogenic transplantation of these cells. These factors come along side with safety of MSCs, ease of isolation, multiple sources and more importantly nonethical constraints on their use. All of this will make MSCs cells of choice for clinical application of tissue regeneration.

We assume that WJ will be one of the major sources of MSCs that will play an important role in tissue engineering in the near future. Actually, this source of MSCs fulfills all advantageous characteristics needed for the clinical setting and one could easily assume that in the next few years, WJ-MSCs will effectively find their own way to be used in several human diseases.

However, many aspects need to be elucidated before MSCs can be efficiently used for regenerative medicine. First, homing of these cells inside human body and how we can target these cells is an issue of extensive research. Second, methods of culturing of these cells need to be not only reproducible but also fulfil GMP to be applied for clinical use. Third, any unknown effects on the human health must be carefully monitored before these cells can be effectively
used in human. Also, these issues will be revealed and resolved within the next few years and will push the whole world to a new era of tissue regeneration as a new modality for treatment of various diseases.

Supplementary
To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/full/10.2217/rme.15.49

Financial & competing interests disclosure
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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research
The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

- Umbilical cord is considered rich source of mesenchymal stem cells (MSCs).
- MSCs can be isolated and expanded from both umbilical cord blood (UCB) and Wharton’s jelly (WJ), the connective tissue surrounding the blood vessels of the umbilical cord.

Results

- WJ-MSCs are more abundant, more homogeneous, more easily isolated and expanded than UCB-MSCs, which are more suitable for clinical settings.
- WJ-MSCs can be better differentiated into insulin producing cells than those of UCB evidenced by both genetic and functional assays.
- WJ-MSCs can better control blood glucose levels in streptozotocin-induced diabetic rats when compared with UCB-MSCs.

Conclusion

- WJ-MSCs are better source of MSCs for stem cell banking and regenerative medicine for diabetes mellitus as compared with UCB-MSCs.

References

Papers of special note have been highlighted as:

- of interest; •• of considerable interest


•• An excellent comprehensive review on the potential use of mesenchymal stem cells (MSCs) for diabetes mellitus (DM) treatment.
11 Chao KC, Chao KF, Fu YS, Liu SH. Islet-like clusters derived from mesenchymal stem cells in Wharton’s jelly of the human umbilical cord for transplantation to control Type 1 diabetes. *PLoS ONE* 3(1), e451 (2008).

•• One of the earliest and highly cited papers highlighting the use of Wharton’s jelly (WJ)-MSCs in Type I DM.
• The method paper used to isolate WJ-MSCs.


• This paper proposed the cellular criteria of MSCs definition as per International Society for Cellular Therapy.


• Among the first papers to provide evidence of Nestin expression in WJ-MSCs.
Kadam SS, Bhonde RR. Islet neogenesis from the constitutively nestin expressing human umbilical cord matrix derived mesenchymal stem cells. Islets 2(2), 112–120 (2010).


Study showing WJ-MSCs as potential treatment of Type 2 diabetes, which adds more value to clinical applications of these cells.


Interesting review highlighting the useful aspect of immunomodulation of MSCs in addition to its tissue regeneration abilities