Original Article

Differentiation of Alginate-Encapsulated Wharton Jelly-Derived Mesenchymal Stem Cells into Insulin Producing Cells

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Abstract

Objective: Insulin insufficiency due to the reduced pancreatic beta cell number is the hallmark of diabetes, resulting in an intense focus on the development of beta-cell replacement options. One approach to overcome the problem is to search for alternative sources to induce insulin-producing cells (IPCs), the advent of mesenchymal stem cells (MSCs) holds great promise for producing ample IPCs. Encapsulate the MSCs with alginate improved anti-inflammatory effects of MSC treatment. This study aimed to evaluate the differentiation of wharton jelly-derived MSCs into insulin producing cells using alginate encapsulation.

Materials and Methods: In this experimental study, we established an efficient IPCs differentiation strategy of human MSCs derived from the umbilical cord's Wharton jelly with lentiviral transduction of Pancreas/duodenum homeobox protein 1 (*PDX1*) in a 21-day period using alginate encapsulation by poly-L-lysine (PLL) and poly-L-ornithine (PLO) outer layer. During differentiation, the expression level of PDX1 and secretion of insulin proteins were increased.

Results: Results showed that during time, the cell viability remained high at 87% at day 7. After 21 days, the differentiated beta-like cells in microcapsules were morphologically similar to primary beta cells. Evaluation of the expression of *PDX1* and *INS* by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) on days 7, 14 and 21 of differentiation exhibited the highest expression on day 14. At the protein level, the expression of these two pancreatic markers was observed after *PDX1* transduction. Results showed that the intracellular and extracellular insulin levels in the cells receiving *PDX1* is higher than the control group. The current data showed that encapsulation with alginate by PLL and PLO outer layer permitted to increase the microcapsules' beta cell differentiation.

Conclusion: Encapsulate the transduced-MSCs with alginate can be applied in an *in vivo* model in order to do the further analysis.

Keywords: Alginate, Diabetes, Insulin, Mesenchymal Stem Cells

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Introduction

Diabetes is one of the metabolic disorders which are caused by impaired insulin secretion, insulin dysfunction, or both. Numerous pathogenic processes contribute to the progression of diabetes, which has a wide range from progressive autoimmune destruction of pancreatic cells toward ultimately insulin deficiency and insulin resistance. Until 2017, 387 millions of people worldwide suffer from diabetes, and that number is expected to reach in to 500 millions by 2030 (1). These statistics have led the international diabetes federation (IDF) in to describe this disease as one of the most serious human health challenges in the 21st century (2).

So far, no certain cure option for diabetes currently exists. At present, taking oral medications and insulin injections are common treatments. Transplantation of islets isolated from the donor pancreas could also be a therapy for diabetes. However, this treatment also has some restrictions, including limited islets required for transplantation, side effects of long-term immunosuppressive drugs, and short-term survival after transplantation. Recently, the application of stem cells that differentiate into pancreatic beta cells has drawn great attention as one of the treatment options for diabetes (3). Some studies suggested that embryonic stem cells, induced pluripotent stem cells (iPCs), bone marrow-mesenchymal stem cells (BM-MSCs) and adipose tissue-MSCs can differentiate into IPCs both *in vitro* and *in vivo* (4). Stem cells with the ability to differentiate into insulin-producing cells (IPCs) are becoming the most promising therapy for diabetes mellitus that reduce the major limitations of availability and allogeneic rejection of beta cell transplantations (5, 6).

Previous studies have reported that IPCs were derived from embryonic stem cells in mice and humans (7, 8). MSCs have potentials for differentiating into various tissues, immunomodulatory effects and the invasiveness of the procedure. Therefore MSCs can overcome the obstacles seen with embryonic stem cells (9).

MSCs are the most important candidates for cell

therapy which are obtained from different sources including BM, adipose tissue, blood, amniotic fluid and umbilical cord of newborns. Recently umbilical cord derived MSCs from Wharton jelly have drawn many attentions because of their differentiation, migration and protective properties compare to other kinds of stem cells. Hu et al. (10) evaluated the application of umbilical cord's Wharton jelly-derived MSCs (WJ-MSCs) for type 1 diabetic patients and obtained promising results. Another study established that transplantation of placenta-derived MSCs for patients with type 2 diabetes was safe, easy, and potentially effective (11).

Alginate hydrogels have demonstrated high applicability as a structure for cell immobilization. Alginate is recognized in properties such as its ability to make hydrogels at physiological conditions, gentle dissolution of gels for cell retrieval, transparency for microscopic evaluation, gel pore network that allows diffusion of nutrients and wastes in addition to its reduced risk of graft failure (12). Encapsulation is a method used to protect implanted cells from immune system attack and it may enhance the survival rate and differentiation of implanted cells by the increased of cytokines secreted by encapsulated cells to the microenvironment (6, 13). Microencapsulation is widely used for encapsulation of cells or bioactive molecules, gene therapy and drug delivery. Hydrogels are the most widely used materials for cell microencapsulation because of their high porosity that leads to high permeability of oxygen, nutrients, and metabolites (14). Alginate widely used for cell encapsulation provides protection of the encapsulated cells against the host's immune system (15). Previous studies demonstrated that MSC encapsulated in alginate could survive locally after implantation *in vivo* (16).

The transplantation of pancreatic islets in immune protective capsules holds the promise as a functional cure for type 1 diabetes (17), about 40 years after the first proof of principal study (18). Gene therapy, as an advanced technology to treat diseases cannot be treated with conventional medicine and can be applied to a wide range of diseases that includes many methods of gene transfer (19). Gene therapy had been approved for diseases such as cystic fibrosis, diabetes, autoimmune diseases, heart diseases Alzheimer's disease, Parkinson's disease, various cancers (20). Gene therapy by viral vector and non-viral transduction may be useful techniques to treat diabetes (21). Insulin generation in MSCs through genetic engineering is a promising therapeutic for patients with diabetes (22). In previous study it was indicated that PDX1-tranduced hBM-MSCs differentiate into IPCs (21). This study aimed to evaluate the differentiation of wharton jelly-derived MScs into insulin producing cells using alginate encapsulation.

Material and Methods

Isolation of MSCs from Wharton jelly

In this experimental study, umbilical cords were collected from healthy full-term deliveries after receiving consent from parents. The collection and using of human biological specimens were approved by the Ethics Committee of the Islamic Azad University-Science and Research Branch (IR.IAU.SRB.REC.1398.214). The Umbilical cords were transferred in serum-free Dulbecco's Modified Eagle Medium/F12 (DMEM/F-12, Hyclone, Logan, UT, USA) and transferred to the laboratory immediately. After washing, the Umbilical cords samples-were cut into 2-3 cm sections, the umbilical vessels removed, and Wharton jelly was collected and minced into pieces. The pieces were plated in tissue culture flasks containing an enzymatic solution of collagenase and hyaluronidase, in DMEM/F-12 medium supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and incubated at 37°C in a humidified 5% CO_2 incubator for 45 minutes to 2 hours. This allows Wharton jelly loosening and separation from the Umbilical cords without complete digestion. After the incubation period, the Umbilical cords pieces are transferred to a new Petri dish or culture flask containing fresh DMEM to remove any remaining enzymes (23).

Flow cytometry analysis

Human MSCs single-cell suspensions were harvested using a 0.05% trypsin/ Ethylenediaminetetraacetic acid (EDTA) solution; after FBS neutralization incubated in blocking buffer [1% FBS in Dulbecco's phosphate-buffered saline (DPBS)] for 30 minutes. Next, 1×10^6 cells were separately incubated for 1 hour at 4°C with an optimal dilution of conjugated antibodies that included anti-CD73-FITC (ab28061), anti-CD45-FITC (ab27287), anti-CD90-FITC (ab11155), anti-CD34-PE (ab157304), and anti-CD105-PE (ab91138), all from Abcam (Cambridge, UK). Flow cytometry experiments were performed with a BD FACSCalibur Flow Cytometer (BD Biosciences) and data analyzed by the Flowing software.

Multilineage differentiation

To confirm the multipotency of WJ-MSCs, osteogenic and adipogenic differentiation were verified with alizarin red and oil red O staining respectively. To induce osteogenesis, WJ-MSCs treated with osteogenic differentiation medium, alpha minimum essential medium (Life Technologies, USA) supplemented with 10% FBS (Gibco, USA), 10 mmol/L β-glycerophosphate (Sigma-Aldrich, USA), 0.1 mmol/L dexamethasone (Sigma-Aldrich, USA) and 50 mmol/L ascorbic acid (Sigma-Aldrich, USA) for 21 day. For adipogenesis, the cells were treated with adipogenic differentiation medium

in alpha minimum essential medium supplemented with 10% FBS, 1 mmol/L dexamethasone (Sigma-Aldrich, USA), 10 mg/mL insulin (Sigma-Aldrich, USA), 0.5 mmol/L isobutyl-methylxanthine (Sigma-Aldrich, USA) and 100 mmol/L indomethacin (Sigma-Aldrich, USA) for 21 day.

Transduction of WJ-MSCs

To transduce *PDX1* using lentivirus system, approximately 1×10^6 MSCs were seeded in 48-well plates. The Lentivector Packaging kit (Invitrogen, USA, K4975-00) including the pPackH1 Packaging Plasmid (mixture of pPACKH1-GAG, pPACKH1-REV, and pVSV-G plasmids, 0.5 µg/µl) and the transfer vector Plenti-Pdx1-PURO (0.5 µg/µl) containing an enhanced *PDX1*, was used to transduce *PDX1* into WJ-MSCs. Virion particles were produced in 293T cells (Invitrogen, Carlsbad, CA, USA) by transfection using the TransIT-2020 Transfection Reagent (Mirus, Madison, WI, USA). The 293T cells were seeded in 75-cm^2 flasks at an initial density of 1.3×10^5 cells/cm^2 with 10 ml of DMEM containing 10% FBS, 50 U/ml penicillin, and 50 µg/ml streptomycin. At 24 hours post-transfection, the media was replaced with fresh DMEM with 2% FBS. The medium was changed every 24 hours for 3 days. The media was removed, pooled, and filtered (pore size: 0.45 µm; Merck Millipore, Rockland, MA, USA), and centrifuged at 50,000 × g for 90 minutes. The resulting pellets were resuspended in serum-free DMEM. The virus titer was determined by transducing 293T cells with the viral preparation and examining *PDX1* expression using polymerase chain reaction (PCR) analysis. The virus titers used in experiments were $1\text{-}2\times10^7$ transducing units/ml.

After virus exposure, transduced MSCs were cultured in serum-containing medium for 5-8 days, and medium was changed every 48 hours. The transduction efficacy was assessed 5 days after transduction. Cells were rinsed with phosphate buffer solution (PBS Gibco BRL, Grand Island, NY, USA) and incubated with 0.5% trypsin/0.2% EDTA (Sigma-Aldrich, USA) for 10 minutes to dissociate the cells. The viability of dispersed cells was evaluated by trypan blue exclusion.

Investigation of differentiation of WJ-MSCs into insulin-producing cells

Quantitative reverse transcriptase-polymerase chain reaction

The expression of pancreatic-specific genes *PDX1* and *INS* was analyzed by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) in differentiated cells on days 7, 14, and 21. MSCs that were not cultured in the differentiating medium containing high glucose DMEM supplemented with 0.5 mmol/L β-mercaptoethanol (Invitrogen, USA, 1% non-essential amino acids (Gibico, UK), 20 ng/ml β-fibroblast growth factor (bFGF, Sigma-Aldrich, USA), 20 ng/ml (EGF, Sigma-Aldrich, USA), 2% B27 (Gibico, UK), 2 mmol/L L-glutamine (Hyclone, USA) , 10 ng/ml β-cellulin (Sigma-Aldrich, USA), 10 ng/ml activin A (Sigma-Aldrich, USA), 2% B27 and 10 mmol/L nicotinamide (Sigma-Aldrich, USA) (7) were used as negative control, and PANC-1 cell line (pancreatic epithelial cells) was used as a positive control (24).

Total cellular RNA was extracted by the TRIzol reagent® (Sigma-Aldrich, T9424) and used for cDNA synthesis with the Revert Aid First Strand cDNA Synthesis Kit (Fermentas, Germany, K1632) according to the manufacturer's instructions. Quantitative RT-PCR was carried out with the SYBR Green Master Mix (Takara Bio, Inc., RR081Q) with a real-time RT-PCR system (Corbett Life Science, Rotor-Gene 6000, Australia). The expression levels of the target genes were calculated using the $2^{-\Delta Ct}$ method with glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) as the internal control for normalization. Primer sequences for target genes are listed in Table 1.

Western blotting

For western blot analysis at the end of treatment, on day 21, the cells were lysed in commercial lysis buffer (Qproteome Mammalian Protein Prep Kit, QIAGEN) according to the manufacturer's protocol. The solubilized protein fractions of each sample (50 µg) from three biological replicates were separated on a 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a PVDF membrane (Amersham Biosciences, USA) by semi-dry blotting (Bio-Rad, USA) using transfer buffer (10 mM $NaCHO_3$, 3 mM Na_2CO_3, 20% methanol). Membranes were blocked with Tris-buffered saline with Tween® 20 (TBST, 20 mM tris-HCl, pH=7.6, 150 mM NaCl, and 0.1% Tween-20) that contained 5% BSA and then incubated overnight with the primary antibody at 4°C. After three times washing with TBST, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody at room temperature for 1 hour. Signals were detected with ECL substrate using Hyperfilm. Protein band intensity was normalized to the level of beta-actin. Each experiment was repeated at least three times.

Encapsulation of WJ-MSCs

The encapsulation of WJ-MSCs was performed as reported by Kanafi et al. (25), with slight modifications. An alginate solution (2% w/v) was prepared by dissolving 2 g of low viscosity alginic acid sodium salt (Low viscosity 100-300 cP, Sigma-Aldrich, USA) in 100 ml of deionized water. The alginate solution was mixed by overnight vortexing. To prepare cell-encapsulated beads, WJ-MSCs at passage 4 were harvested and a cell density of 5×10^5 was mixed in 1 ml of alginate solution. The alginate solution was transferred to a 5 ml syringe (22 Gauge) and then extruded dropwise into an ice-cold 100 mM calcium

chloride ($CaCl_2$, Sigma-Aldrich, USA) solution. The droplets were left 10 minutes in the $CaCl_2$ solution for polymerization. Cell-encapsulated microcapsules were then transferred to 35 mm tissue culture dishes containing 1.5 ml DMEM medium supplemented with FBS. The WJ-MSC beads were incubated at 37°C in 5% CO_2 incubator for 72 hours and then used for further experiments.

Decapsulation

Three days after encapsulation, microcapsules containing WJ-MSCs were washed by PBS twice, and 10 ml of decapsulation solution (EDTA, 50 mM and HEPES, 10 mM in PBS, Sigma-Aldrich, USA) was added, then the beads were incubated at 37°C for 10 minutes. The cells were pelleted by centrifugation at 3000 rpm for 10 minutes. This cell pellet was used for RNA isolation or protein extraction.

Cell viability assessment

Assessment of WJ-MSCs viability was performed using the MTT assay. The cells at a density of 2×10^5/well were inoculated into a 96-well plate. The WJ-MSCs were divided into transduce and transduced groups. The plates were placed in an incubator at 5% CO_2 at 37°C overnight. WJ-MSCs were transduced with Plenti-Pdx1-PURO lentivirus (5×10^9 TU/ml) at 200 MOI based on the results of transfection efficiency, and WJ-MSCs in the untransfected group received an equivalent dose of PBS. Cells in one of the four plates were incubated with the MTT solution at 7, 14, and 21 days after transfection. After 4 hours, the medium was removed, and 150 µl of dimethylsulfoxide (DMSO) added to each well. Absorbance was measured at 570 nm using an ELISA reader (Biochrom Anthos 2020, UK).

Insulin assay and response to glucose

Insulin level in the medium was measured by human insulin ELISA kit (Millipore, Billerica, MA, USA) according to the manufacturer's instructions. Total protein in the medium was measured by the BCA assay using fresh culture medium as a blank. To determine the cell response to glucose at different concentrations, the insulin levels were evaluated with different glucose concentrations (0, 5.5, 15 and 25 mM). 1×10^6 cells were initially incubated for 3 hours in glucose-free Krebs-Ringer bicarbonate buffer (KRB). This was followed by incubation for 1 hour in 3.0 mL of KRB containing 0, 5.5, 15, or 25 mM glucose concentrations. The supernatant was collected at the end of each incubation period. The collected samples were using the ELISA assay (26). We evaluated the insulin level in positive control group to confirm the insulin ELISA kit.

Statistical analysis

All experiments were conducted in at least three independent repeats and performed in the same passage. Statistical analysis was performed using GraphPad Prism 5.02 (GraphPad Software, Inc, USA). Comparisons between groups were performed by one-way analysis of variance (ANOVA) followed by the Tukey post-hoc test. The independent t test analysis was carried out to identify statistical differences between the two observations. The difference between data was considered to be significant at $P<0.05$.

Results

Derivation and characterization of WJ-MSCs

MSCs derived from human umbilical cord's Wharton Jelly, human WJ-MSCs had fibroblastic-like phenotype (Fig.1A), the cells were small and fusiform at the first passage. After third passage, the cells seem fully expanded with many cytoplasmic processes. To confirm the mesenchymal identity, the expression of MSC-specific markers was examined. Results from flow cytometry showed that the expression (%) of these markers including CD105, CD90 and CD73 in WJ-MSCs were 97.4, 96.70 and 95.3, respectively. While hematopoietic specific markers such as CD34 and CD45 did not have significant expression in these cell populations. These results confirm that the isolated cells from human umbilical cord's Wharton jelly are MSCs (Fig.1B). WJ-MSCs are determined as multipotent stem cells that are able to differentiate into specific lineages like osteoblastic and adipocytic. Thus, the osteogenic differentiation assay was performed to examine the differentiation ability of isolated MSCs into these two lineages. Intracellular lipid droplets staining using oil red- O showed the adipogenesis of WJ-MSCs. While in the undifferentiated WJ-MSCs, these observations were absent. Alizarin staining demonstrated the formation of calcium oxalates on the differentiated MSCs, which was not detected in the undifferentiated cells. These findings confirmed the characterization of cells as WJ-MSCs and indicate that the MSCs have potential to differentiate into these lineages (Fig.1C).

Differentiation of MSCs derived from Wharton jelly into IPCs

To investigate whether transduction of WJ-MSCs with *PDX1* leads to their differentiation into IPCs *in vitro*, we used the lentiviral vector to transfer the *PDX1* gene into MSCs (Fig.2A). For this, WJ-MSCs were cultured in 6-well plates at 1×10^6 cells/well (at passage number 3), when the confluency were reached 70-80%, transduction was performed (Fig.2B). After selecting the transduced cells using puromycin (at a concentration of 2 mg/ml),

these cells were cultured in serum-free newly culture medium for 21 days (Fig.2C). To evaluate the transduction efficiency of target cells, we evaluated the expression of beta cell-specific genes *PDX1* and *INS* by qRT-PCR on days 7, 14 and 21 of culture (Fig.3A-C). We found that the expression levels of *PDX1* and insulin genes in the PDX1-transduced group were higher than the negative control groups. Whereas, the difference between PDX1-transduced group and positive control groups (pancreatic cell) was not significant. On day 7, *PDX1* and *INS* showed the lowest expression, and on day 14, exhibited the highest expression. We also examined the expression of these genes at the protein level by Western blotting; the expression of these proteins was examined 21 days after *PDX1* transduction of WJ-MSCs. The results showed that PDX1 protein level was increased in transduced group (Fig.3D).

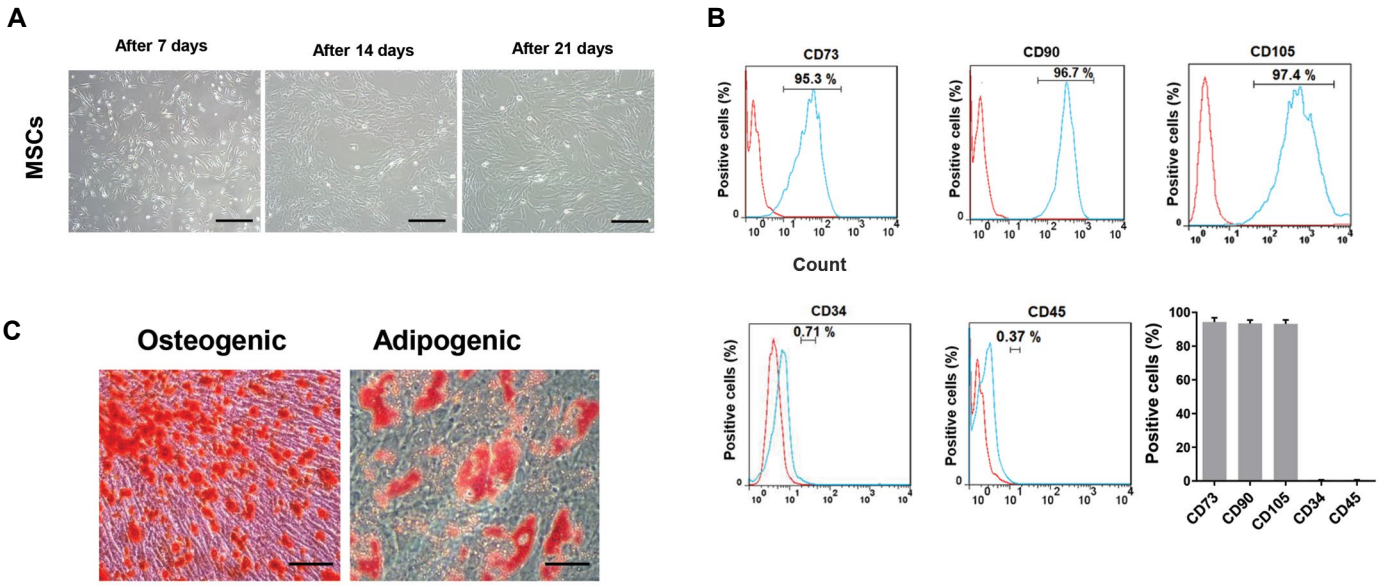

Fig.1: Isolation of MSCs from Wharton jelly, culture, and identification. **A.** Culture of WJ-MSCs during 21 days (scale bar: 100 µm). **B.** Evaluation of CD markers by flow cytometry. The WJ-MSCs expressed CD105, CD90, and CD73 but they expressed CD34 and CD45 at very low level. Each cell treatment was assayed on three technical replicates on three different samples of WJ-MSCs. **C.** Alizarin red staining after 21 day of culture in osteogenic medium indicated the osteogenic differentiation potential of WJ-MSCs (scale bar: 50 µm). Oil red staining after 21 day of culture in adipogenic medium showed the adipogenic differentiation potential of WJ-MSCs. Data for each day represent mean cells number and error bars show standard error of the mean (SEM) of triplicate experiment (n=3). MSC; Mesenchymal stem cells and CD; Cluster of differentiation.

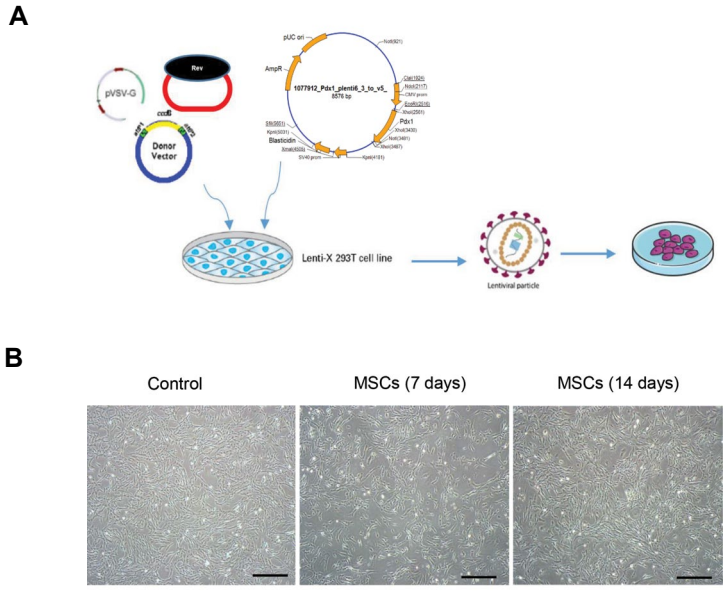

Fig.2: Differentiation of WJ-MSCs into IPCs. A. Schematic summary of transduction pdx1-planti in cultured WJ-MSCs. B. Morphology of WJ-MSCs were transduced with the PDX1 gene using the Lentiviral vector system (scale bar: 200 µm). WJ-MSCs; Wharton jelly-derived mesenchymal stem cells and IPCs; Induce insulin-producing cells.

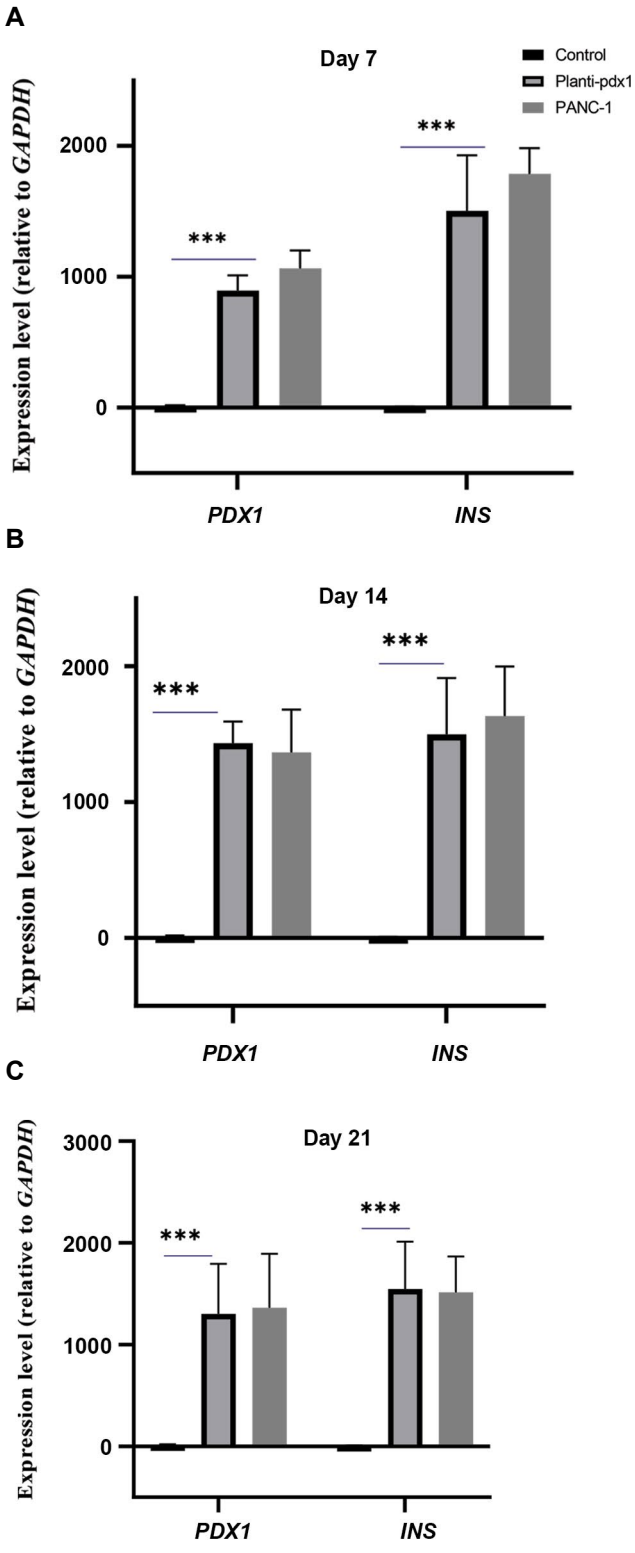

Fig.3: Investigation of differentiation of WJ-MSCs into IPCs. **A.**, **B.**, **C.** Expression of pancreatic-specific genes *PDX1* and *INS* was analyzed by qRT-PCR in differentiated cells on days 7, 14 and 21. MSCs that were not cultured in the differentiating medium were used as negative control, and PANC-1 cell line was used as positive control. Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was considered as the housekeeping control. Each experiment was conducted with in triplicate (n=3). **D.** Western blot analysis for PDX1 and its phosphorylated form (pPDX1) in MSC-Pdx1. Values represent mean and error bars show standard error of the mean (SEM) of triplicate experiment (n=3). ***; P<0.001, WJ-MSCs; Wharton jelly-derived mesenchymal stem cells, IPCs; Induce insulin-producing cells, and qRT-PCR; Quantitative reverse transcriptase–polymerase chain reaction.

Viability, and intra- and extracellular insulin levels in encapsulated PDX1-tranduced MSCs

In order to evaluate the effect of cell encapsulation by alginate hydrogel, the cell viability was evaluated by MTT assay on days 7, 14 and 21 after encapsulation (Fig.4A, B). The results showed 98% viability in the group that the cells were decapsulated immediately after encapsulation. After 7 days of cell encapsulation, cell viability remained high at about 87%. However, this rate was decreased to 79% following 14 days due to repeated passages. The viability of transduced-MSCs significantly increased following encapsulation by alginate on day 21. At the following examinations, to evaluate the functionality of encapsulated differentiated cells carrying *PDX1*, intra- and extracellular insulin levels were measured on day 14 using ELISA assay. Results showed that the intracellular and extracellular insulin levels in the MSCs receiving *PDX1* is higher than the control group at concentration of 5.5, 15 and 25 ng/mg insulin protein (Fig.5A, B).

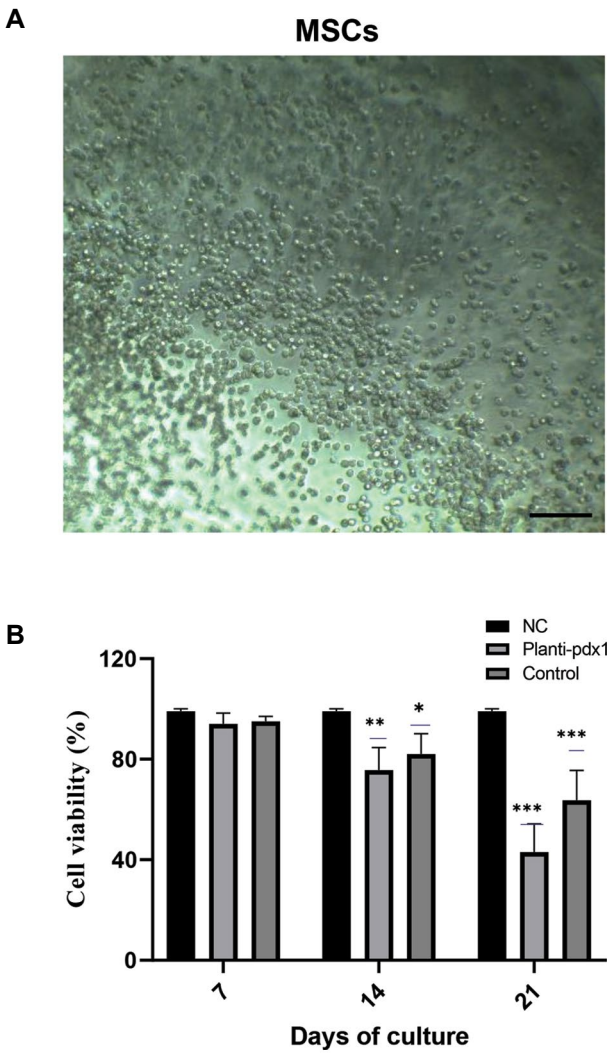

Fig.4: Encapsulated MSCs in alginate hydrogels. **A.** The microscopic image of the capsules containing MSCs showed the uniform distribution of the cells within hydrogel. The average diameter of the capsules is 650 µm (scale bars: 50 µm). **B.** Percentage of viable cells evaluated by MTT assay, after 7, 14 and 21 days (n=3). MSCs; Mesenchymal stem cells, NC; Negative control, *; P<0.05, **; P<0.01, and ***; P<0.001.

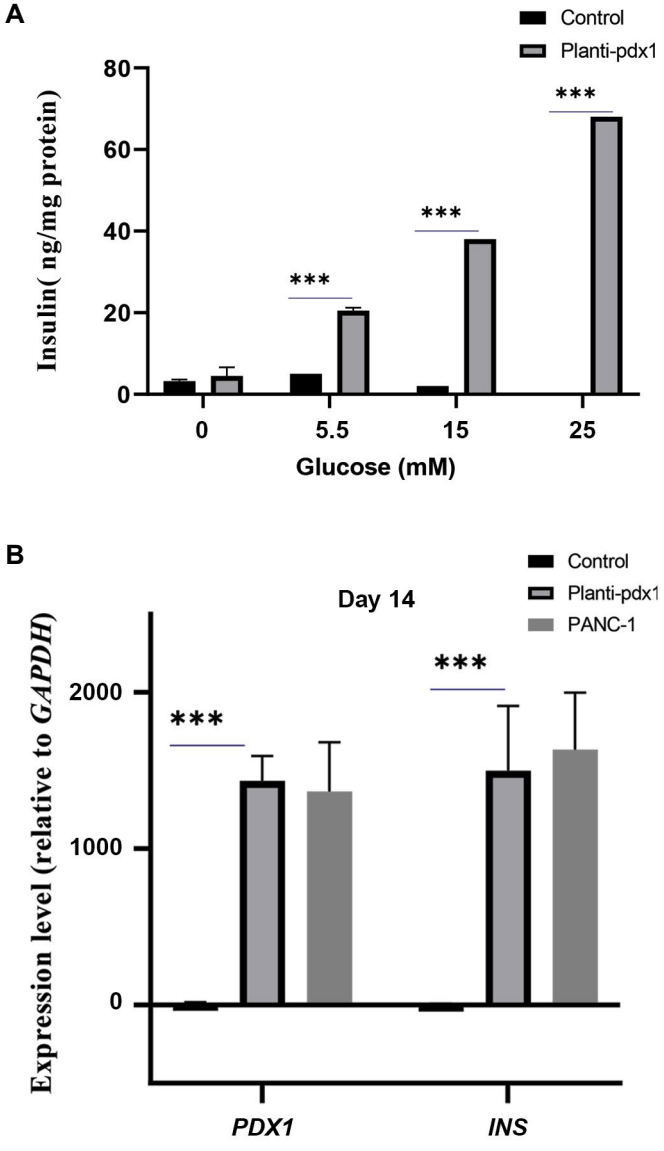

Fig.5: Insulin assay and response to glucose. **A.** The expression of level insulin protein in the differentiated cells compared to the undifferentiated cells. Conversion factor for insulin: 1 µg=23 mU/L, 1 mU=0.6 pmol/L. **B.** Concentrations of intracellular insulin protein content in differentiated cells compared to undifferentiated cells. Conversion factor for insulin: 1 µg=23 mU/L, 1 mU=0.6 pmol/L (n=3). Control group; Cells without encapsulation and transduction. ***; Significant.

Discussion

The prevalence of diabetes is steadily increasing worldwide, making it one of the most challenging health issues of the 21st century (2). Therefore, it is necessary to search new effective treatment strategies aimed at recovering lost IPCs and inhibiting autoimmune destruction of endocrine progenitor cells (27). Also, results of a meta-analysis by El-Badawy and El-Badri showed that MSCs could be beneficial in patients with type 1 and type 2 diabetes (28). Differentiation of BM-MSCs and adipose tissue-MSCs into IPCs provides a new and promising strategy to reconstitute pancreatic endocrine function (26). In line with previous studies, our results showed, WJ-MSCs Were differentiated into IPCs. Therefore, WJ-MSCs are a promising source for applying in diabetes due to their availability, low cost, and immune-modulatory properties. To produce IPCs from WJ-MSC origin, two methods of indirect and direct differentiation are used (29, 30). Indirect differentiation is performed using chemicals (e.g., nicotinamide and growth factors) and direct differentiation is based on genetic manipulation (31). We showed that transfer of *PDX1* gene with lentiviral vector caused the differentiation of WJ-MSCs into IPCs, and confirming obtained results, an increase in the expression of pancreatic-specific genes such as *PDX1* and insulin was observed. The results of our study were comparable to the findings of Rahmati et al. (32), in which the transfer of *PDX1* gene to mouse MSCs with lentiviral method led to the differentiation of these cells to IPCs. In a study by Soltanian et al. (33), assembling three-dimensional (3D) pancreatic organoids (containing human embryonic stem cell-derived PDX1-positive pancreatic progenitors, MSCs, and endothelial cells) implanted into the peritoneal cavity of immunodeficient mice where it remained for 90 days. Their results indicated that 3D organoids developed more vascularization and a higher number of insulin-positive cells and improvement of human C-peptide secretions. Lima et al. (34) through overexpression of three beta cell-specific genes *PDX1,* Neurogenin-3 *(NEUROG3),* and V-maf musculoaponeurotic fibrosarcoma oncogene homolog A (*MAFA*) by adenoviral vectors, trans-differentiated pancreatic exocrine cells into beta-like cells both morphologically and functionally. In this study, we indicated that PDX1-tranduced MSCs group differentiate into IPCs.

Besides, for a durable treatment of insulin-dependent diabetes mellitus, it is crucial to establish a functional system that, in addition to supporting the insulin secretion in response to different levels of glucose, protecting from immune system. The viability and function of differentiated WJ-MSCs is the most important issue in the use of these cells in diabetes therapy. In this study, alginate hydrogel was used for encapsulation of the cells to avoid a declined survival. One important characteristic of alginate is its very limited inherent cell adhesion and cellular interaction, that is an advantage for cell encapsulation applications (12). The technique to cell immobilization, particularly pancreatic islet cells, in calcium alginate matrices was developed by Lim and Sun (18). By coating the alginate gel bead with polycations like PLL, PLO, or chitosan, the strength of the surface coating as well as the capsule porosity can be controlled (12). In this study, for production of alginate droplets (microcapsules), the viscous solution of alginate was mixed with the cells and then these were stabilized by treatment with polycationic polymers. A combination of PLL and PLO was applied as polycation in order to improve the strength of outer surface of microcapsules. The essential requirements for cell culture i.e. porosity, stability and permeability were reached by alginate in MSCs differentiation into IPCs (35). Consistent results by some studies were reported that pancreatic islet, ESC- and iPSC-derived IPCs encapsulated using alginate could maintain the viability both *in vivo* and *in vitro* (36).

The differentiated WJ-MSCs which were encapsulated by alginate, showed an increased cell viability, however this value was decreased to 79% after 14 days. Thickening of the outer layer by polycationic polymers may also cause the insufficient nutrient and oxygen consumption, which results in a significant reduction in cell viability on day 21. However, despite declined cell viability, insulin secretion levels did not decrease on days 7, 14 and 21. The high number of viable cells in viscous alginate capsules can also lead to high insulin secretion Encapsulation MSCs with alginate enhance the survival rate and differentiation of transduced MSCs (37).

Similar results have been obtained from other studies. In a recent study, alginate solution improved survival and maintenance of cell functionality in encapsulation of BRIN-BD11 beta cell line, and also the expression of *INS* was increased by 66% (38). Recently, Kuncorojakti et al. (3) evaluated the encapsulation of human dental pulp-derived stem cells (hDPSCs)-derived IPCs by alginate and pluronic F127-coated alginate. Obtained results showed that alginate and alginate combined with pluronic F127 preserved hDPSCs viability and allowed glucose and insulin diffusion in and out. In hDPSC-derived IPCs maintained viability for at least 14 days and sustained pancreatic endoderm marker (NGN3), NKX6.1, MAF-A, ISL-1, GLUT2 as pancreatic islet markers, and intracellular pro-*INS* and *INS* expressions for a 14-days period. In another study, differentiation of WJ-MSCs into IPCs using a lentiviral system containing the GFP reporter gene and its transmission to diabetic NOD mice showed an elevated level of serum insulin and an improved glucose tolerance. Mice treated with WJ-MSCs-GFP had significantly lower blood sugar and higher survival rates than control mice (39). Results from a recent study by De Mesmaeker et al. (40) showed that encapsulation of porcine islet cells by alginate hydrogel in microsphere form enabled long-term glycaemic control in immune-compromised mouse model of diabetes. This intracapsular functional beta cell mass formation involved beta cell replication, significant increasing number, and maturation toward human adult beta cells.

Conclusion

Our results showed that the differentiation of WJ-MSCs into Insulin Producing Cells is increased in PDX1-tranduced MSCs group. The *INS* level in encapsulated PDX1-tranduced MSCs with alginate was increased compared to the control group. Therefore due to the ability of WJ-MSC in amelioration fibrosis, modulation inflammation and enhancement vascular growth, MSCs could offer a promising treatment option for patients with endocrine disorders.

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Authors' Contributions

Z.P.; Participated in study design, data collection and evaluation, drafting and statistical analysis. S.K., N.H.R.; Were involved in data analysis and interpretation. S.A.; Was involved in conducting the experiments, manuscript proof, administrative and financial support. All authors read and approved the final manuscript.

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