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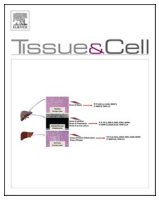

The impact of diabetic glucose concentration on viability and cardiac differentiation of mesenchymal stem cells

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ABSTRACT

Introduction: Hyperglycemia may be a stumbling block for delivery of regenerative benefits of mesenchymal stem cells (MSCs) to diabetic patients with cardiovascular diseases. Our study aims to assess the viability and cardiac differentiation potential of MSCs after being exposed to diabetic glucose concentration.
Methods: MSCs were extracted from rat bone marrow. Cells were characterized based on morphology, differentiation potential, and expression of mesenchymal specific markers. MTT assay was done to evaluate the viability of MSCs after treatment with different glucose concentrations. Case group was MSCs treated with diabetic concentration of glucose versus cells treated with PBS as the control group. Growth curve and population doubling time were calculated in both groups. Expression of GATA4 and troponin, as the early and late markers during cardiac differentiation, were measured following 5-azacytidine exposure.
Results: Proliferated cells at passage three had fibroblastic-shape, was able to differentiate into adipocytes or osteocytes, and expressed CD73 and CD90. MSCs viability was gradually decreased by increasing glucose concentration. Irrespective of nicotine concentration, three-day exposure imposed more severe detrimental effects on viability compared with one-day treatment. Proliferation rate of the MSCs was lower in the case group, and they need more time for population doubling. Expression of both cardiac markers were downregulated in the case group at day three. However, their expression became higher at day seven.
Conclusion: Diabetic glucose concentration inhibits normal proliferation and cardiac differentiation of MSCs. This effect should be considered in stem cell therapy of cardiovascular patients who are concurrently affected by hyperglycemia, a common comorbidity in such individuals.
Why carry out this study?

- Stem cell therapy has opened a promising window to reduce great burden imposed by cardiovascular disease.
- Despite substantial benefits observed in early studies, clinical translation of such treatment approaches have been hampered.
- Hyperglycemic condition may be one of the hurdles responsible for reduced beneficial effects of stem cell therapy in cardiovascular patients.

What was learned from the study?
Findings:

- of our study revealed that diabetic glucose concentration inhibits normal proliferation and cardiac differentiation of mesenchymal stem cells.
- Since diabetes is one of the common comorbidity in patients with cardiovascular diseases, glycemic status of such patients should be considered in the time of stem cell transplantation.

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- Moreover, these patients may need some pretreatments or augmented cells for transplantation in order to observe maximum benefits.

1. Introduction

Atherosclerotic complications and ischemic events are the major contributors to global health problems. Notably, these health challenges are closely intertwined with type 2 diabetes (Pinhas-Hamiel and Zeitler, 2007), which inherently induces macrovascular and microvascular pathologies (Grundy, 2012; Fowler, 2011). In fact, heart apparatus is severely damaged from chronic hyperglycemia. Although routine cell function and respiration need glucose (Sawangmake et al., 2014), high concentration of this vital substance causes impairments in the function and proliferation of endothelial and mesangial cells (Fadini et al., 2005; Lin et al., 2008). Considering lack of efficient functional regeneration in adult heart after injury alongside limitations of the current remedies, stem cell therapy has gained much attention during the last years. Stem cells and progenitors are the ones that perform tissue reconstitution in response to cell insults (Alison and Islam, 2009). In particular, stem cells have the potential to restore injured cardiovascular tissues (Giordano et al., 2007).

Although the benefits of stem cell therapy have been confirmed in a wide variety of diseases, unsuccessful experiences are still concerning (Lechner et al., 2004; Meza-Zepeda et al., 2008). One failure is this fact that survival and proliferation of transplanted cells to a substantial number and an appreciable effect is not performed. One previously ignored possibility is the hyperglycemic condition of the recipient. Diabetes is among the usual pathologic conditions in cardiovascular patients, who are candidates of stem cell therapy (Stolzing et al., 2006; Li et al., 2007), and this may be the one of the fundamentals for ineffective stem cell therapy. Intriguingly, short term exposure to high glucose concentration do not alter proliferation and production of growth factors in the MSCs (Weil et al., 2009). The mystery of inefficient stem cells in terms of survival and differentiation potentials remain perplexed if such issues lasts unaddressed.

With great plasticity, tissue-resident mesenchymal stem cells (MSCs) has sparked as a promising and safe therapeutic tool for regeneration after ischemic events (Kim and Ahn, 2012; Telukuntla et al., 2013), especially in the context of cardiovascular system (Kang et al., 2015). However, it was shown that diabetic stress is a strong risk factor that declines the therapeutic benefits of the MSCs following cardiac injury (Kim et al., 2014; Molgat et al., 2014). So, scrutinizing the underlying reasons of reduced MSCs' function is a priority. To substantiate, maternal diabetes attenuates expression of transcription factors essential for cardiac specification (Gu et al., 2016; Wang et al., 2015), which emphasizes the adverse impact of high glucose on cardiogenesis in early embryonic development (Yang et al., 2016). There are few studies that have explored the effect of high glucose concentration on viability and cardiac differentiation of the MSCs, which are the popular stem cell type in clinical trials of cardiovascular patients.

The current investigation tries to elucidate the basic reasons responsible for reduced effectiveness of MSC therapy in smoker patients with CVD. We hypothesize that nicotine adversely affects the biological activity of the MSCs.

2. Materials and methods

2.1. Establishing the bone marrow-derived MSCs at P3

Three male rats (4–6 weeks old, 110–140 g weight) were obtained from animal house affiliated with University of Medical Sciences. In order to have a homogenous cell culture devoid of biological differences, a MSC pool was prepared from aggregation of bone marrow cells extracted from three rats. Briefly, rats underwent euthanization by combined IP injection of ketamine (300–360 mg/kg) and xylazine (30–40 mg/kg). In sterile condition, tibias and femurs of both sides were exposed and cleaned from adjacent tissues. They were placed in PBS supplemented by penicillin/streptomycin. After cutting the epiphysis, bones were flushed by DMEM culture medium from both ends. The extracted cell suspension was collected, and subjected to centrifugation (5 min., 1200 rpm, room temperature). Discarding the supernatant, cell pellet was dissolved in 1 ml. of fresh culture medium, added to a prefilled T25 culture flask, and transferred to a humidified incubator (5% Co_2) at 37C. After 24 h., culture medium was replaced, and medium replacement was repeated every 3–4 days. The culture flask became confluent after 10–15 days (Passage 0). At this time, cells were treated by trypsin/EDTA in order to disengage from the flask surface. Cell suspension was centrifuged again and the resulting cell pellet was seeded into two T25 culture flask in a similar way as previously mentioned. Growth and proliferation of these cells yielded MSCs at passage one. The whole procedure was repeated until obtaining MSCs at passage three (Fathi et al., 2022a).

-Characterization of the MSCs based on morphology, differentiation ability, and expression of specific markers

Morphology of the cells was monitored from the first to the third passage. In each step, cell images were taken by the aid of an invert microscopy. MSCs at P3 were exposed to adipogenic culture medium containing HAMS F-12, FBS (15%), L-glutamine (0.2 mM), L-ascorbic acid (100 mM), indomethacin (200 μM), and dexamethasone (100 nM). Following 21 days of humidified incubation under 5% Co_2 condition at 37C, cells were fixed and stained by Oil red O dye. For osteogenic induction, cells were treated by a mixture including low-glucose DMEM medium, ascorbate 2-phosphate (0.05 mM), glycerophosphate (10 mM), dexamethasone (100 nM), FBS (10%), and antibiotic (1%). After incubation for 21 days, the cells were fixed and stained by Alizarin red dye. Regarding expression of specific mesenchymal markers, a confluent T25 culture flask at P3 was prepared. Treatment of the cell culture with trypsin/EDTA produced a suspension that was further centrifuged. The cell pellet was subjected to RNA extraction (Rnx Plus Solution, CinnaGen Co.). The quality and quantity of extracted RNA were examined by NanoDrop. RNA was then transcribed into cDNA by AddScript cDNA Synthesis Kit (add bio, South Korea). Using mesenchymal specific primers (Table 1), PCR (Taq DNA Polymerase Master Mix RED, Ampliqon, Denmark) was performed under defined time and temperature cycles. Binding of PCR products to DNA safe stain in agarose gel electrophoresis make them visible under the illumination of a UV lamp by a gel documentation system (Jahromi et al., 2017).

2.2. MTT assay

In order to assess the viability of the MSCs exposed to different glucose concentrations, MTT assay was carried out. MSCs at P3 were prepared in three 96-well plates (5 000 cells in each well) for analysis after one, two, and three days. Cells were incubated for 24 h. following seeding, and then, different concentration of glucose (from 1 to 10^7 ng/ml, dilution factor of 10) were added to each well. In the corresponding

Table 1
Primer sequences of mesenchymal specific primers.

Markers	Forward primer	Reverse primer
CD73	TGCATCGATATGGCCAGTCC	AATCCATCCCCACCGTTGAC
CD90	GACCCAGGACGGAGCTATTG	TCATGCTGGATGGGCAAGTT

days, culture medium was discarded, and MTT dye (20 µlit, 5 mg/ml) dissolved in sterile PBS was added to each well. After 4 h. of incubation, plate was centrifuged, and the supernatant was omitted. According to the instruction, a definite volume of DMSO was added to each well, and following complete solubilization, absorbance was measured at 750 nm wavelength (Karimi-Haghighi et al., 2022). This procedure was done in triplicates. According to the MTT findings and based on blood concentration of glucose in patients with diabetes (126 mg/dL or 7.0 mM), a glucose concentration of was chosen for upcoming experiments. So, the case group was defined as the MSCs treated by glucose at 8.5 mM concentration, and the MSCs in the control group was exposed to the similar volume of sterile PBS.

2.3. Growth curve and population doubling time

MSCs at P3 were prepared at two 24-well plates for the case and control groups. After 24 h. of incubation, three wells in each group were considered, their culture medium were discarded, and the cells were washed by PBS. Trypsin/EDTA treatment followed by centrifugation yielded cell pellet, which was further suspended by adding a specific volume of culture medium. A definite volume (for example 7 µlit) of the cell suspension was mixed with the same volume of trypan blue dye. Number of cells in this mixture were determined by using a Neubauer chamber. Considering dilution factor, mean number of cells in three wells was designated as the number of cells in day one. These procedures were repeated for eight consecutive days. This whole step was done in triplicates (Ghobadi et al., 2018).

-Quantitative measurement of cardiac specific markers in the MSCs treated by glucose after cardiogenic induction

In four T75 flasks, MSCs at P3 were prepared: two were allocated for the case group and two for the control group. Each group was intended to be assessed in days three and seven. The MSCs were let to be proliferated and upon reaching confluent, all flasks were firstly treated by 5-azacytidine, a well-known substance that is used for cardiogenic differentiation, for 24 h. Then, cells in each group were treated by defined culture medium of the case and control groups, either glucose or PBS. In days three and seven, the cells were harvested and subjected to RNA extraction and cDNA synthesis as previously mentioned. qPCR (RealQ Plus 2x Master Mix Green, Ampliqon, Denmark) was performed with cardiac specific primers of GATA4 and troponin (Table 2) in a defined time and temperature cycles. This procedure was done in triplicates (Fathi et al., 2020).

2.4. Statistical analysis

Statistical Package for Social Sciences (SPSS, V16) was used for analysis. Comparison of the case and control groups was done by either independent sample t test or ANOVA. Differential gene expression was assessed by $2^{\sim\Delta\Delta Ct}$ method. *P* value of <0.05 was considered statistically significance.

3. Results

3.1. Characterization of the MSCs

In order to be assure about the identity of proliferated cells at P3, certain criteria including morphology, differentiation capability, and expression of mesenchymal specific markers should be examined. After extracting of cells from the bone marrow, they were cultured and subcultured until reaching P3. Although the cell pool demonstrated different morphologies at earlier passages, typical appearance of the MSCs in the form of fibroblastic- or spindle shape cells became prevalent during the later subcultures (Fig. 1). Differentiation capability was tested in adipogenic and osteogenic exclusive media. After being exposed for three weeks, the cells were stained by specific dyes to examine the generation of adipocytes or osteocytes (Fig. 2). The final step for characterization of the MSCs is examining the expression of mesenchymal specific markers. As shown (Fig. 3), expression of CD73 and CD90 were confirmed through reverse transcriptase PCR. However, It would be better if we examined the expression of mesenchymal specific markers along with hematopoietic markers by flow cytometry (Fathi et al., 2022b).

Table 2
Primer sequences of markers used in quantitative PCR.

Markers	Forward primer	Reverse primer
GATA4	TGATGGATGGAAGAAGAT	GTGATGAAGACAAGGAAG
Troponin	CAGAGTATCCACAACCTA	CAGTTCCATCTATTTCCAA
GAPDH	AAACCCATCACCATCTTCCA	CACGACATACTCAGCACCA

3.2. MTT assay

MTT assay was done in order to evaluate the impact of different glucose concentrations on the viability of MSCs and also, to determine the glucose concentration of interest for next steps. Viability of the MSCs gradually became lower by increasing the concentration of glucose. The highest viability was seen in 2.5 µM while 2.5×10^{6} µM glucose concentration produced the lowest viability. In similar glucose concentration, viability of the MSCs was higher in the first day compared with the third day, which shows a time-dependent viability of glucose-treated MSCs (Fig. 4).

3.3. Growth curve and population doubling time (PDT)

Growth rate of the MSCs exposed to glucose was screened for eight consecutive days (Fig. 5). Cells in the case group were at the lag phase for one day like their peers in the control group. Both groups started the exponential proliferation from day two. However, the extent of proliferation was remarkably lower in the case cells than the control counterparts during this period. The decreasing trend in the growth rate was seen after the 5th day in both groups, which was followed by plateau phase. During plateau (from day 6–8), number of cells in the case group remains lower. In total, this finding shows that glucose inhibits the MSCs from normal proliferation and growth (Fig. 5). Indeed, time needed for population doubling in the case group was about 1.6 times more than the control group (Table 3). This indeed demonstrates the inhibitory effect of glucose on proliferation of the MSCs, which in turn reflects in higher time needed for increasing population of cells.

3.4. Appearance of the glucose-treated MSCs at days three and seven

The MSCs culture in the case group had less density compared with the control peers. Also, organization of the cells in the control group seemed to be healthier and well-constructed than the case cells. Fibroblastic- or spindle shape cells, the typical appearance of the MSCs, were more in the control group (Fig. 6).

-Differential expression of cardiac genes at days three and seven after cardiogenic induction

GATA4 and troponin, as two specific cardiac markers that are expressed successively during differentiation, were differently expressed in the MSCs of the case and control groups. Expression ratio of GATA4 was 0.26 in the case/control groups at day three while it became 1.18 at day seven. Similarly, expression ratio of troponin was 0.41 in the case/control groups at day three, and reversed to 1.26 at day seven (Fig. 7).

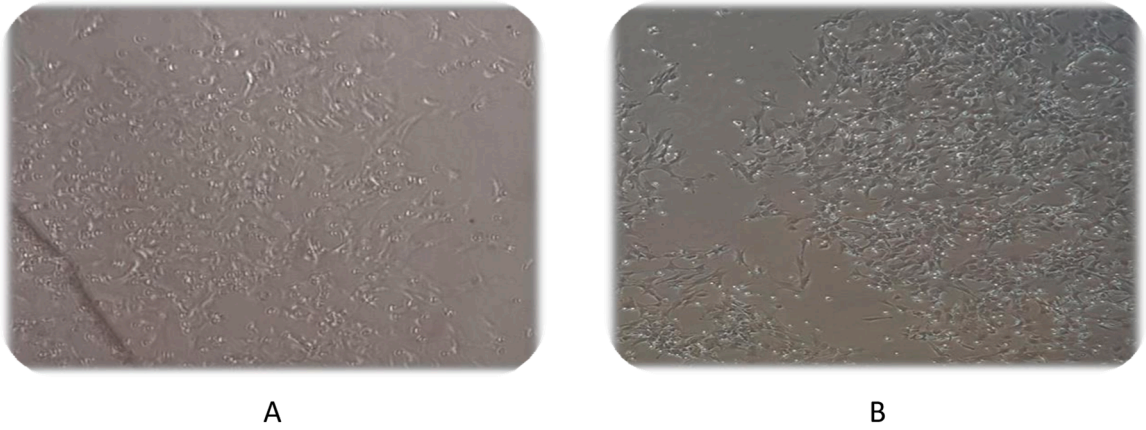

Fig. 1. A: Cells with different morphologies at earlier passage. B: Cells with fibroblastic- or spindle, shape morphology are dominant at passage 3. (Magnification=360X).

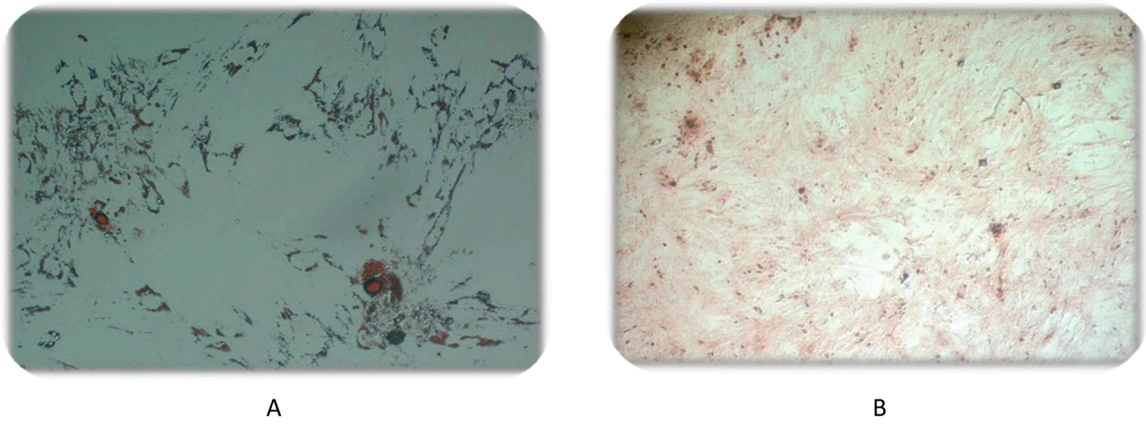

Fig. 2. Differentiation of mesenchymal stem cells into adipocytes (empty with a thin rim of, cytoplasm close into the basal lamina) in A (Oil red O staining) and -into osteocytes (irregular, spherical shape) in B (Alizarin red staining). (Magnification=360X).

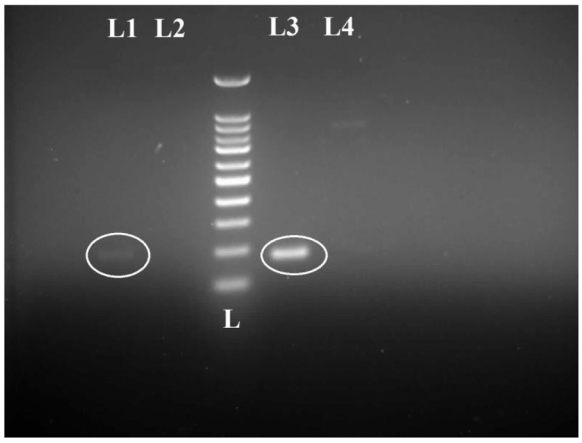

Fig. 3. Expression of CD73 (208 bp) and CD90 (197 bp) by MSCs (lanes 1 and 3). Lanes 2 and 4 were, hematopoietic progenitors (negative control) without expression of mesenchymal markers. Ladder: 100 bp. bp=base pairs.

4. Discussion

Cardiovascular disease (CVD) has undermined the health of millions throughput the world (Roth et al., 2020). A well-established correlation is documented between CVD and diabetes (Cramer et al., 2010). Type II diabetes not only imposes detrimental effects on cardiac tissue and vascular remodeling, but also deteriorates the function of various cell types including stem cells (Ding and Triggle, 2005; Efimenko et al., 2015). For instance, hyperglycemia impairs hematopoietic function of the bone marrow (Ferraro et al., 2011), diabetes changes chemokine expression of the MSCs (Kočí et al., 2014), and high glucose concentration leads to premature senescence, genomic instability, and telomere alterations in culture conditions (Stolzing et al., 2006; Parsch et al., 2004; Estrada et al., 2013). These effects merit consideration especially in patients with CVD, who are the frontline candidates of stem cell therapy since hyperglycemia is a common comorbidity is such patients (Dhanasekaran et al., 2013). It was shown that diabetic condition declines the efficiency of stem cell therapy in cardiovascular trials (Kim et al., 2014; Molgat et al., 2014). One study reported that MSCs exposed to high glucose stress fail to generate sufficient vascularization in the infarcted myocardium (Kang et al., 2015), and also, lose their potential to alleviate the complications relevant to type II diabetes (Meza-Zepeda et al., 2008). Moreover, this aspect of diabetic pathology is of eminent importance for designing and implementation of new therapies (Cramer et al., 2010).

MSCs are known as one of the most suitable cell types to restore the lost function after injury (Assmus et al., 2002; Orlic et al., 2001). Other than safety and possibility for autologous transplantation, the ameliorating effects of the MSCs in cardiac repair attribute to inflammation reduction, and cell death inhibition through releasing a bunch of soluble factors (Kim and Ahn, 2012; Telukuntla et al., 2013). Advantages of the MSCs for cell therapy are also derived from homing, immune modulation, and wound healing properties (Wakao et al., 2012). MSCs show

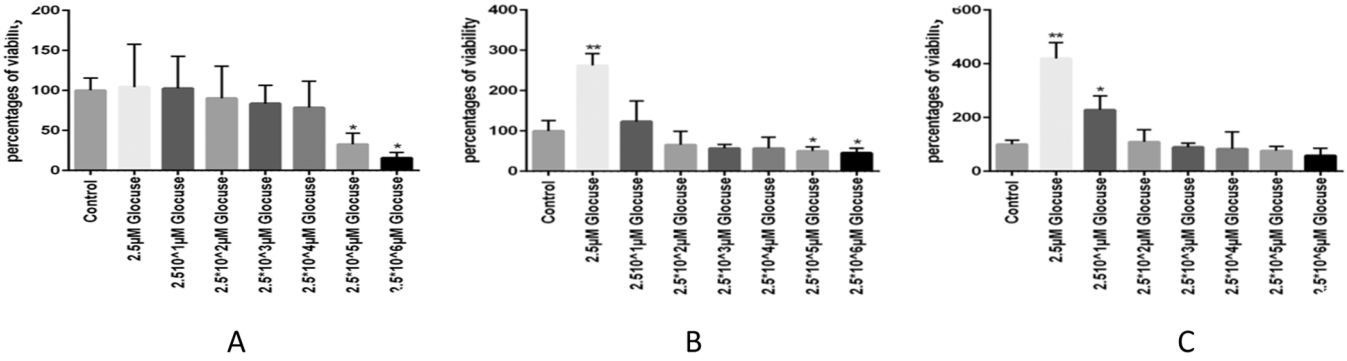

Fig. 4. MSCs viability in percentage after one- (A), two- (B), and three (C) day(s) of exposure to, different concentrations of glucose (from 2.5 μM to 2.5×106 μM). Asterisk shows statistical significance compared with the control group.

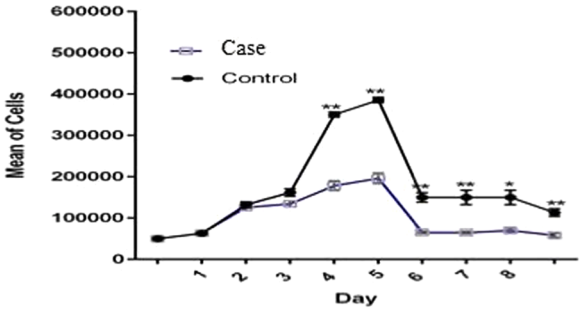

Fig. 5. Growth curve of the case group in comparison to the control group during eight consecutive days. Asterisk shows statistical significance compared with the control group.

Table 3
Population doubling time (hours) of the cells in the case and control group.

Days	Control group	Case group
1	9.26	9.54
2	16.66	18.37
3	18.02	23.84
4	23.22	30.40
5	43.32	76.40
6	51.98	92.55
7	60.65	100.58
8	81.16	136.44

site-specific differentiation potential, and adapt new functions in different biomolecular contexts (De Miguel et al., 2012; Ankrum and Karp, 2010). They release antiapoptotic factors and promote neo-angiogenesis. All these effects improve reoxygenation resulting in regeneration of the infarcted tissue (Figeac et al., 2014; Zhang and Xu, 2014). By reduction in scar size, substantial improvement in cardiac function is also emerged.

However, many unsuccessful instances of stem cell therapy have postponed its clinical translation. One barrier is diabetes and high glucose condition that deregulate MSCs' characteristics such as survival, proliferation, and differentiation, as the necessary elements of their regenerative ability (Dhanasekaran et al., 2013). Hence, it is necessary to conduct experiments in order to reveal any association between high glucose condition and the functionality of the MSCs. To the best of our knowledge, this is a maiden attempt to specifically respond to this perplexity regarding the effects of diabetic glucose concentration on proliferation and cardiac differentiation of bone marrow-derived MSCs.

MSCs need an approximate glucose concentration of 5.5 mM in culture condition for maintenance (Dhanasekaran et al., 2013). Aging is promoted and proliferation is inhibited following treatment of the MSCs by a high glucose condition (Zhang et al., 2017). A 16.5 mM glucose concentration inhibited proliferation of rat bone marrow-MSCs (Gopalakrishnan et al., 2006) and rat mandibular osteoblasts (Ma et al., 2011). Our study showed that glucose concentration of 8.5 mM prevent MSCs from normal proliferation, which was revealed by both growth curve and population doubling time. In 16.5 mM glucose condition, the proportion of cells at G1 is remarkably increased because expression of Ckd4, which is responsible for progression of the cell cycle from G1 to the S phase, is prevented (Ma et al., 2011; Abbott et al., 2004). Induction of apoptosis in high glucose concentration is observed as early as 120 h via activation of caspase genes (Stolzing et al., 2006; Dezawa et al., 2005). However, there are also studies that report MSCs resistance to the short-term exposure of high glucose concentration (Weil et al., 2009). MSCs derived from both diabetic and non-diabetic individuals that were treated with high glucose demonstrated lower cell replication and elevated cell senescence. However, these deteriorating effects were more pronounced in cells from diabetic patients (Cramer et al., 2010).

Increased proliferation rate of bone marrow-MSCs were seen after exposure to 50–100 nM nicotine for 7 days (Shen et al., 2013). Another study declared that elevated proliferation was not occurred at 1 μM to 100 μM nicotine concentration in alveolar BM-MSCs, concentration of 1 and 2 mM may increase proliferation, and those of >5 mM did not cause optimal proliferation (Kim et al., 2012). Such dose-dependent decrease in proliferation rate beside increased apoptosis was also evident in human umbilical cord-MSCs due to elevated level of oxidative stress and arrest in G0 cell cycle (Li et al., 2018). Significant decrease in the proliferation potential of the MSCs isolated from chronic smokers should be considered as well in donating cells in order to avoid transplantation with attenuated proliferative capacity (Harrell et al., 2022).

High glucose concentration also produced drastic effects on differentiation ability of the MSCs. Advanced glycation end products interfere with adipogenic-, chondrogenic-, and osteogenic differentiation of the MSCs (Kume et al., 2005). Adiponectin, an antidiabetic, anti-inflammatory, and antiatherogenic factor, is downregulated in the MSCs isolated from diabetics (Rasouli and Kern, 2008; Deepa and Dong, 2009). Osteogenic and chondrogenic differentiation capacities are also considerably lower in the MSCs derived from diabetic patients compared with nondiabetic peers. However, adipogenesis was declared to be higher in the MSCs from patients with diabetes (Cramer et al., 2010).

It was shown that nicotine negatively affect osteogenic differentiation activity of the MSCs (Ng et al., 2015). This effect is also dose-dependent via binding of nicotine to the subunits of nicotinic acetylcholine (Zhou et al., 2013). Detrimental effects of cigarette smoke was also reported on chondrogenic differentiation capacity of the MSCs (wahl 2016). In contrary, one study demonstrated positive effects of nicotine on chondrogenic potential of the MSCs derived from adipose tissue (Roux et al., 2013). These contradictory findings may be explained by different origins of the MSCs and dissimilar nicotine concentrations. Moreover, it seems that adipogenic differentiation of the

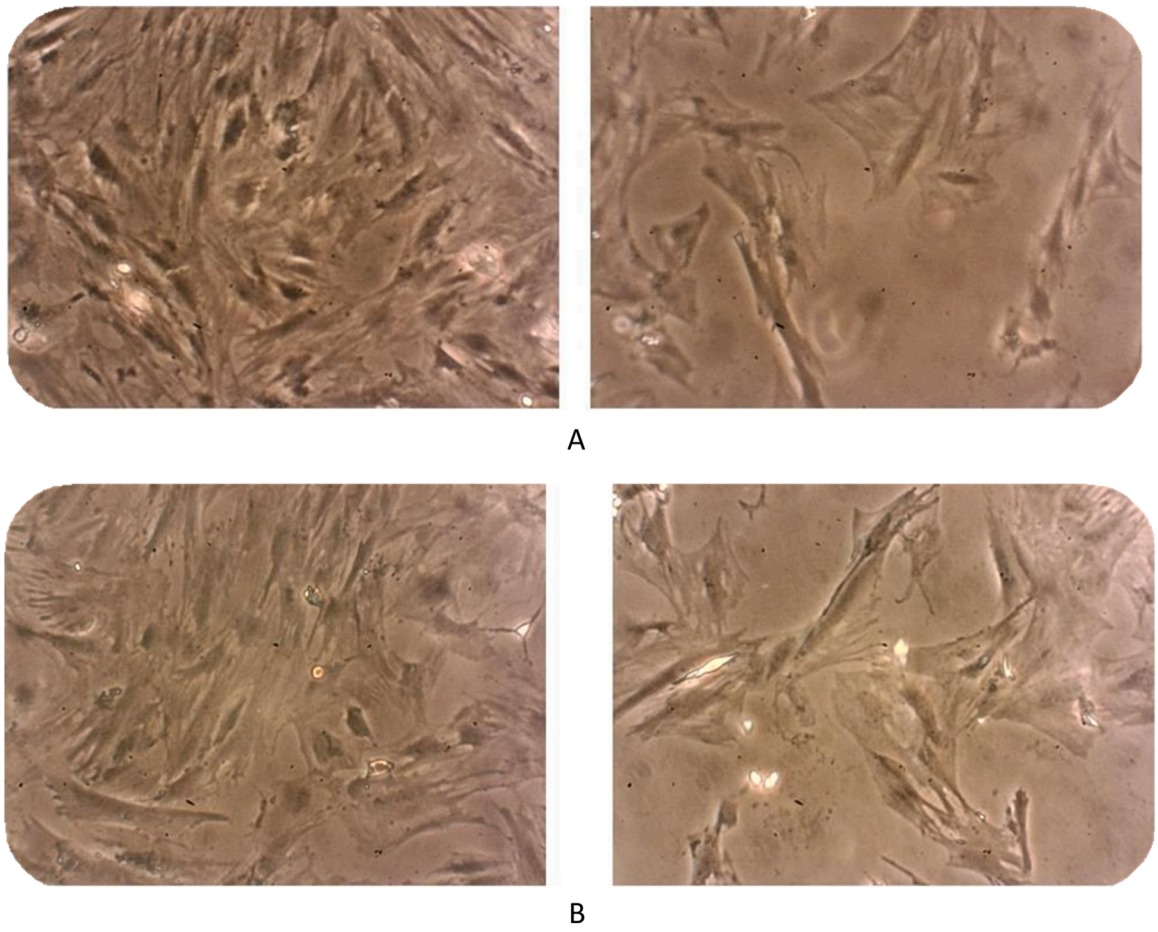

Fig. 6. Appearance of the MSCs at day three (up) and seven (down) in the control (left) and case (right) groups. Note to the higher density and healthier organization of the MSCs in the control versus case group (Magnification=360X).

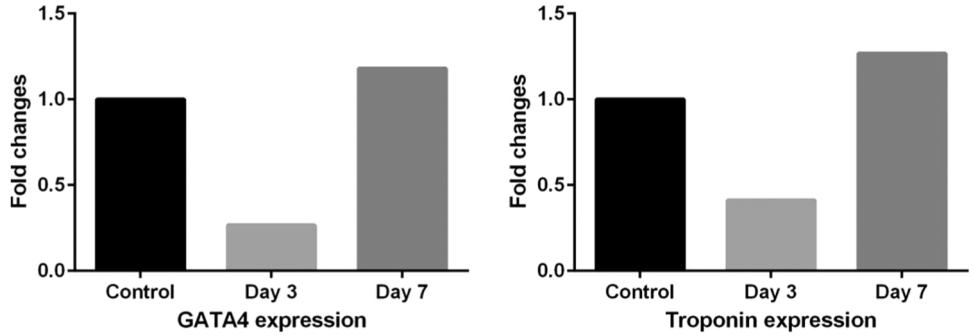

Fig. 7. Differential expression of cardiac genes between case and control groups at days three and seven. Fold changes were calculated via 2-ΔΔCt.

MSCs was not changed upon exposure to cigarette smoke extract (Wahl et al., 2016). This possibly shows that signaling pathways governing different differentiation potentials different.

Regarding cardiogenic differentiation of glucose-treated MSCs, our findings show that both early and late cardiac specific markers were downregulated after a three-day exposure. However, expression of these markers were increased at day seven, which possibly relates to the increased cell population, and decreased influence of glucose accordingly, combined with cardiogenic impact of 5-azacytidine. Paradoxically, a 25 mM glucose concentration augmented the efficiency of cardiac differentiation toward generation of beating cardiac muscle while propensity of neurogenic differentiation is inhibited (Sawangmake et al., 2014). This demonstrates heterogeneity in cellular differentiation feedbacks even to a definite glucose concentration (Sawangmake et al., 2014; Mochizuki et al., 2011). Expression of numerous genes, important in the pathogenesis of diabetes, is altered in the MSCs from diabetic patients. The interesting point is that this alteration is occurred following being exposed to high glucose concentration for only five days, which is definitely not comparable to the chronic exposure of cells to high glucose microenvironment in patients with diabetes (Cramer et al., 2010).

Thousands of genes that are regulated in a temporal and precise manner govern heart development (Wamstad et al., 2012). Initial proliferation, differentiation, and maturation of cardiomyocytes are tightly controlled by a highly conserved network (Drab et al., 1997). Firstly, mesodermal precursor cells enter into a precardiac stage (Bondue and

Blanpain, 2010; Bondue et al., 2011). Expression of Mesp1 followed by NKX2.5 and TBX5 is combined with GATA4 upregulation leading to the activation of cardiac structural genes (Mummery et al., 2012). NKX2.5, which is the earliest marker during cardiac differentiation in vertebrate embryos (Yang et al., 2016), along with GATA4, are suppressed by high glucose. High glucose condition further downregulates mature cardiomyocyte markers (Yang et al., 2016). Unfavorable sequels of high glucose during heart development are mediated through cellular stress in terms of oxidative stress and endoplasmic reticulum stress (Gu et al., 2016; Wang et al., 2015).

5. Conclusion

Proliferation and cardiac differentiation of the MSCs are declined after exposure to diabetic glucose concentration. The impact of high glucose condition on regenerative characteristics of stem cells should be considered as an influential and determining feature in cardiovascular candidates of cell-based therapies.

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CRediT authorship contribution statement

Maryam Gheisari: Writing – review & editing, Investigation, Data curation. **Shadi Nosrati:** Writing – review & editing, Investigation, Data curation. **Mahintaj Dara:** Writing – review & editing, Methodology, Investigation, Data curation. **Shahrokh Zare:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Iman Razeghian-Jahromi:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Samaneh Zolghadri:** Writing – review & editing, Methodology, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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NA

Compliance with ethics guidelines

All experiments were performed in accordance with relevant guidelines and regulations. Approval was granted by the Ethics Committee of Azad University.

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