


Preconditioning of Human Decidua Basalis Mesenchymal Stem/Stromal Cells with Glucose Increased Their Engraftment and Anti-diabetic Properties

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Abstract

BACKGROUND: Mesenchymal stem/stromal cells (MSCs) from the decidua basalis (DBMSCs) of the human placenta have important functions that make them potential candidates for cellular therapy. Previously, we showed that DBMSC functions do not change significantly in a high oxidative stress environment, which was induced by hydrogen peroxide (H₂O₂) and immune cells. Here, we studied the consequences of glucose, another oxidative stress inducer, on the phenotypic and functional changes in DBMSCs.

METHODS: DBMSCs were exposed to a high level of glucose, and its effect on DBMSC phenotypic and functional properties was determined. DBMSC expression of oxidative stress and immune molecules after exposure to glucose were also identified.

RESULTS: Conditioning of DBMSCs with glucose improved their adhesion and invasion. Glucose also increased DBMSC expression of genes with survival, proliferation, migration, invasion, anti-inflammatory, anti-chemoattractant and antimicrobial properties. In addition, DBMSC expression of B7H4, an inhibitor of T cell proliferation was also enhanced by glucose. Interestingly, glucose modulated DBMSC expression of genes involved in insulin secretion and prevention of diabetes.

CONCLUSION: These data show the potentially beneficial effects of glucose on DBMSC functions. Preconditioning of DBMSCs with glucose may therefore be a rational strategy for increasing their therapeutic potential by enhancing their engraftment efficiency. In addition, glucose may program DBMSCs into insulin producing cells with ability to counteract inflammation and infection associated with diabetes. However, future *in vitro* and *in vivo* studies are essential to investigate the findings of this study further.

Keywords Placental DBMSCs · Glucose · Cellular functions · Gene expression

This paper is dedicated to late “Professor Mohammad Abumaree”, who was the main architect of this study. We will continue to be inspired by his dedication and profound passion for the stem cell research at KAIMRC. May you rest in peace.

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1 Introduction

Mesenchymal stem/stromal cells (MSCs) are isolated from many human adult organs, including the placenta [1–3]. MSCs have multipotent differentiation potential [1–3] and possess immune-modulatory properties [4–7], which are essential for tissue restoration. Therefore, MSCs are

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considered attractive potential therapeutic agents to treat human diseases including diabetes, hypertension, and atherosclerosis [8–10]. In these inflammatory diseases, the environment is characterized by high levels of oxidative stress and inflammation. Consequently, for successful use of MSCs in these diseases, MSCs must maintain their normal functional activities to repair injured tissues in hostile microenvironments associated with oxidatively stress and inflammation. MSCs that cannot resist the toxic environment, are likely to have reduced therapeutic potential [11].

Recently, we reported the isolation and phenotypic characteristics of MSCs from the maternal decidua basalis tissue (DBMSCs) of human term placenta [1]. Human pregnancy is a condition where the maternal tissues and circulation are exposed to high levels of oxidative stress. DBMSCs in the maternal tissue of decidua basalis are a vascular microenvironment (i.e. niche), and are continuously exposed to high levels of oxidative stress products in the maternal circulation [12]. As a result, DBMSCs are conditioned to resist oxidative stress, as previously reported [13]. In a recent study, we showed that DBMSCs survive the harsh oxidative environment induced by high concentrations of H_2O_2 , and that preconditioning of DBMSCs with H_2O_2 improved their functional activities [14]. In addition, that preconditioning of DBMSCs with H_2O_2 modulated their expression of genes with important cellular functions [14]. We also reported that DBMSCs protect endothelial cells functions from the damaging effects of both H_2O_2 and monocytes [15, 16]. Therefore, preconditioning of DBMSCs appears to be a rational approach for increase the efficiency of stem cell therapies associated with inflammatory diseases. In this study, we examined the functional responses of DBMSCs to another oxidative stress mediator; glucose. We exposed DBMSCs to high level of glucose and their phenotypic and functional properties were then assessed. We found that DBMSCs survived the harsh environment provided by high level of glucose, and that preconditioning of DBMSCs with glucose increased important functions, including adhesion and invasion. In addition, preconditioning of DBMSCs with

glucose enhanced their expression of genes associated with various cellular functions including survival, proliferation, migration, invasion, immune modulation and microbial clearance. Glucose also increased DBMSC expression of B7H4, an immune protein with ability to inhibit T cell proliferation. Finally, DBMSC expression of genes involved in insulin secretion and prevention of diabetes was also modulated by glucose. These data indicate that glucose increases functions associated with engraftment of DBMSCs, and induced beneficial phenotypic changes in DBMSCs. We conclude that DBMSCs are potential candidate for the treatment of diabetes through a mechanism involving the reduction of inflammation and the secretion of insulin to lower glucose levels. However, more studies are essential to confirm these findings *in vitro* and *in vivo*.

2 Materials and methods

2.1 Ethical approval and the collection of tissues (human placentae and umbilical cord tissues)

This study was approved by the institutional review board (Reference # IRBC/246/13) at the King Abdulla International Medical Research Centre (KAIMRC). Human placentae and umbilical cord tissues were obtained from term, uncomplicated and healthy pregnancies (pregnant women have no medical problems, such as gestational diabetes or any other types of diabetes) after written consent from the donors. All clinical and experimental techniques in this study were conducted as per the guidelines and regulations of the KAIMRC. Placental tissues were immediately processed.

2.2 Isolation and culture of DBMSCs and HUVEC (human umbilical vein endothelial cells)

DBMSCs were isolated from the decidua basalis which remains attached to the maternal side of the human term placenta after delivery, while HUVEC were isolated from umbilical cord veins as previously described by us [1, 16].

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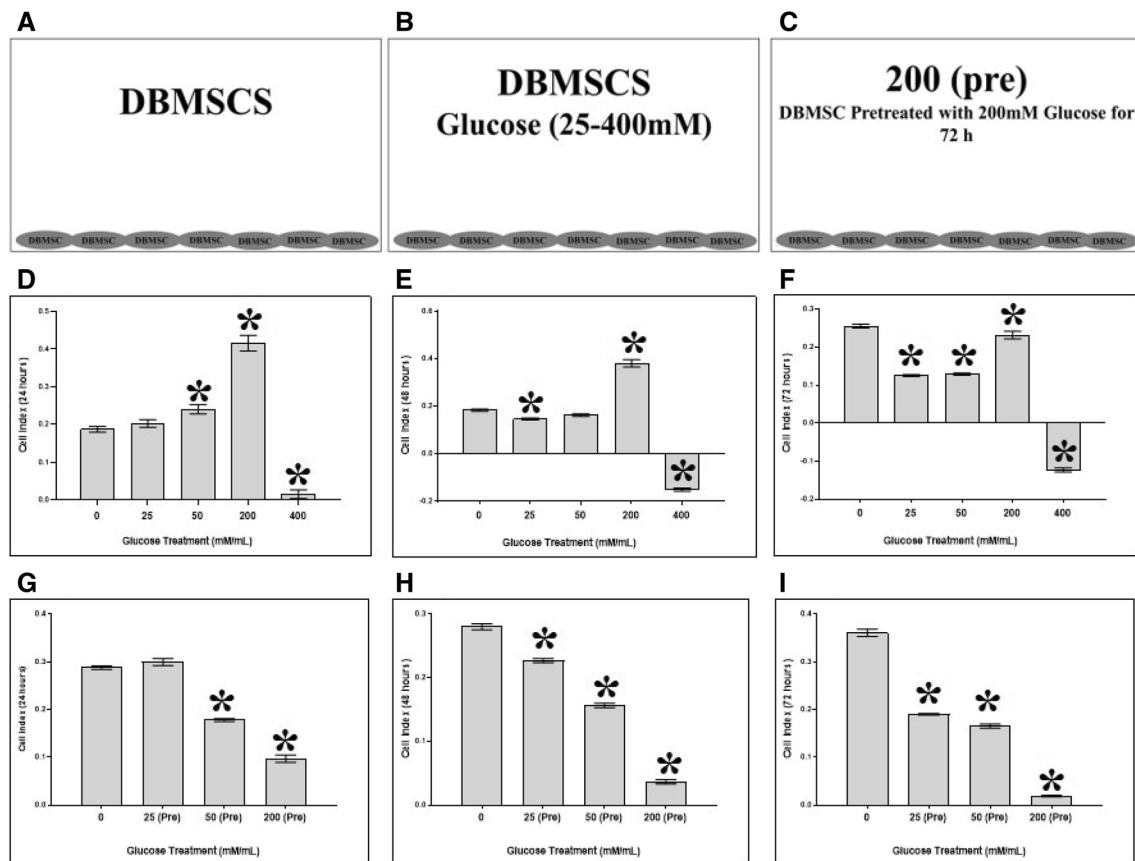


Fig. 1 DBMSC proliferation groups. **A** Group 1 consisted of DBMSC cultured alone in a complete DBMSC culture medium. **B** Group 2 consisted of DBMSC cultured with different concentrations (25–400 mM) of glucose in a complete DBMSC culture medium. **C** Group 3 consisted of DBMSC pretreated with 200 mM glucose for 72 h [200 (pre)], harvested and then re-cultured alone in a complete DBMSC culture medium. **D** DBMSCs were seeded in a 16-well plate (E-Plate 16). The culture plates were then placed in the xCELLigence system at 37 °C in a cell culture incubator, and DBMSC cell index was then monitored. DBMSC proliferation in response to different glucose concentrations by the xCELLigence system. As compared to untreated DBMSCs, DBMSC proliferation was unchanged at 25 mM glucose ($p > 0.05$) but significantly increased at 50 and 200 mM glucose and then significantly reduced

at 400 mM glucose, after 24 h in culture. **E** At 48 h in culture, and as compared to untreated DBMSCs, DBMSC proliferation was unchanged at 50 mM glucose ($p > 0.05$) but significantly increased at 200 mM glucose and then significantly reduced at 25 and 400 mM glucose. **F** At 72 h in culture, and as compared to untreated DBMSCs, DBMSC proliferation significantly increased at 200 mM glucose but was significantly reduced at 25, 50 and 400 mM glucose. **G–I** The reversibility of DBMSC proliferation in response to glucose. DBMSCs were initially cultured with 200 mM glucose for 72 h and their proliferation was then determined using the xCELLigence system. At 24–72 h in culture, and as compared to untreated DBMSCs and DBMSC-treated with 200 mM glucose [200 (I)], the proliferation of DBMSC pretreated with 200 mM glucose [200 (pre)] significantly reduced

DBMSCs were cultured in a complete DBMSC culture medium [DMEM-F12 medium containing 10% MSCFBS (Mesenchymal Stem Cell certified fetal bovine serum, catalogue number 12-662-011, Life Technologies, Grand Island, NY, USA), and antibiotics (100 µg/mL streptomycin and 100 U/mL penicillin)], while HUVEC were cultured in a complete endothelial cell growth medium (Catalogue number PCS-100-041TM, ATCC, Manassas, VA, USA). Cells (DBMSCs and HUVEC) were incubated at 37 °C in a humidified atmosphere containing 5% CO₂ and 95% air (a cell culture incubator). DBMSCs (passage

3) and HUVEC (passages 3–5) of a total of 30 placentae and umbilical cords, respectively, were used in this study.

2.3 DBMSC proliferation and adhesion in response to glucose

DBMSC treatment groups consist of three groups as described in supplementary Table 1 and illustrated in Fig. 1A–C. The xCELLigence system (RTCA-DP version; Roche Diagnostics, Mannheim, Germany) was used to evaluate the adhesion and proliferation of HUVEC as we previously described [15–18]. Briefly, 2×10^4 DBMSCs

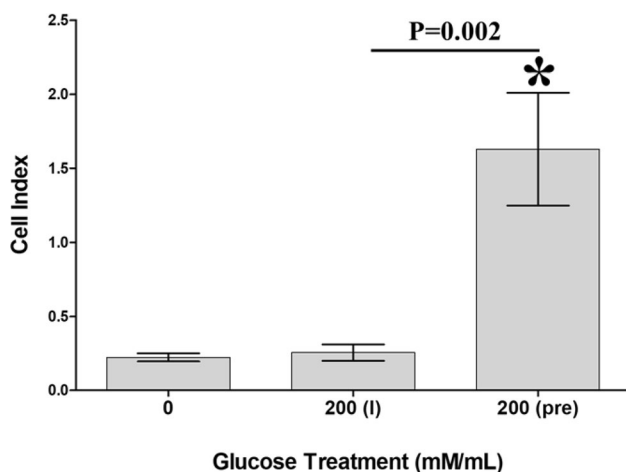


Fig. 2 DBMSCs were cultured with 200 mM glucose [200 (I)] and their adhesion was then determined using the xCELLigence system. As compared to untreated DBMSCs, the treatment with 200 mM glucose had no significant effect on DBMSC adhesion at 2 h in culture ($p > 0.05$) while the adhesion of DBMSC pretreated with 200 mM glucose for 72 h was significantly increased at 2 h as compared to untreated DBMSCs, and DBMSC cultured with 200 mM glucose [200 (I)]. Each experiment was performed in triplicate and repeated with five independent DBMSC (passage 3) preparations. * $p < 0.05$. Bars represent standard errors

were seeded in a complete DBMSC culture medium (as described above) containing different concentrations (25–400 mM) of glucose (Prince Care Pharma Pvt. Ltd, India) in 16-well culture plates (Catalogue number 05469813001, E-Plate 16, Roche Diagnostics). The culture plates were then placed in the xCELLigence system at 37 °C in a cell culture incubator and the DBMSC cell index was then monitored. The data for cell adhesion (at 2 h) and proliferation (24–72 h) was determined as previously described [15–18]. DBMSC viability was determined by Trypan blue exclusion. Each experiment was performed in triplicate and repeated with five independent DBMSC (passage 3) preparations.

2.4 DBMSC migration in response to glucose

DBMSC migration was examined using the xCELLigence system and 16-well plates (Catalogue number 05665825001, CIM-16, Roche Diagnostics GmbH) as we described previously [15–18]. DBMSC treatment groups consist of three groups as described in supplementary Table 2 and illustrated in Fig. 3A–C. Briefly, 2×10^4 DBMSCs were seeded in the upper chamber, and the migration of cells was then monitored by the xCELLigence system [15–18]. The data were then expressed as a cell index value at 24 h. DBMSC migration with 30% FBS and without FBS served as positive and negative controls,

respectively. Each experiment was performed and repeated as described above.

2.5 DBMSC invasion under the effect of glucose

We evaluated the effect of glucose on the ability of DBMSCs to invade through a monolayer of endothelial cells using the xCELLigence system. Briefly, 2×10^4 HUVEC seeded in a complete endothelial cell growth medium in a 16-well culture E-Plate (as described above) until cells reached a growth plateau (20 h). Different treatments of DBMSCs (supplementary Table 3) were then added to the monolayer of endothelial cells. At 10 h, the data for the invasion recorded and expressed as a cell index (mean \pm standard error). The rate of cell invasion was determined by calculating the normalized cell index at pausing time (20 h) of HUVEC growth.

2.6 Gene expression by real-time polymerase chain reaction (RT-PCR)

DBMSC expression of 84 genes related to Human Oxidative Stress (Catalogue # PAHS-065ZD, Qiagen, Hilden, Germany) was identified using our previously published method [1, 14, 15, 18]. Total RNA was extracted from DBMSCs pretreated with 200 mM glucose for 72 h, and cDNA was then synthesized and used in a QuantiTect Primer Assay (Qiagen, Hilden, Germany). The real-time polymerase chain reaction (RT-PCR) was performed in triplicate on the CFX96 real-time PCR detection system (BIO-RAD, Hercules, CA, USA), and the data was then analysed as previously described [1, 14, 15, 18]. The $\Delta\Delta^{-2}$ values were then calculated to express the results as fold changes. The relative expression of internal controls (house-keeping genes) were used as provided in the kit. Experiments were performed in triplicate and repeated three times using DBMSCs prepared from three independent placentae.

2.7 Flow cytometry

DBMSCs (1×10^5) were stained with antibodies for ICAM-1, IL-12, and B7H4 for 30 min and then flow cytometry was performed as previously described [1]. Negative controls were cells stained with FITC or PE-labelled mouse IgG isotype antibody.

2.8 Statistical analysis GraphPad Prism 5 was used to analyze data using non-parametric tests

(Mann–Witney U and Kruskal–Wallis). Data were deemed statistically significant if $p < 0.05$.

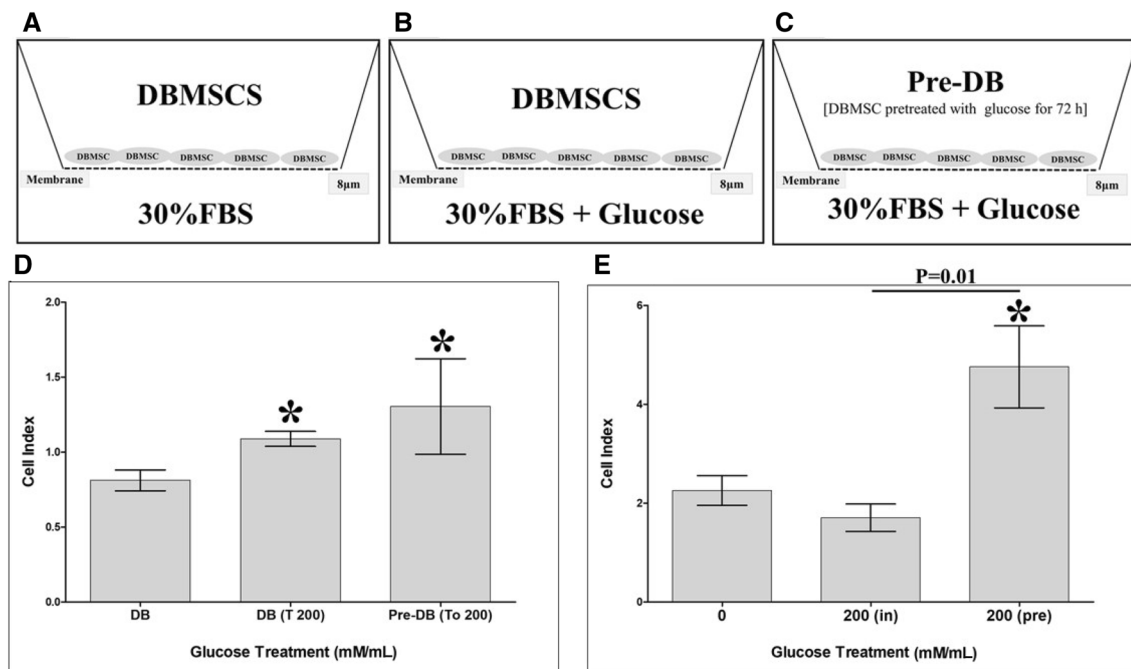


Fig. 3 DBMSC migration groups. **A** Group 1 consisted of DBMSCs cultured alone in the upper chamber. **B** Group 2 consisted of DBMSCs cultured alone in the upper chamber while 200 mM glucose was added to the lower chamber. **C** Group 3 consisted of DBMSCs pre-treated with 200 mM glucose for 72 h (200(pre)) harvested and then re-cultured alone in the upper chamber while 200 mM glucose was added to the lower chamber. **D** DBMSCs were seeded in DBMSC serum free medium in the upper chamber of the CIM migration plate while DBMSC culture medium containing 30% FBS was added to the lower chambers. At 24 h, DBMSC [DB (T 200)] migration in response to 200 mM glucose significantly increased as compared to untreated DBMSCs (DB). The migration of DBMSCs pretreated with 200 mM glucose for 72 h (Pre-DB) in response to 200 mM glucose

[Pre-DB (To 200)] significantly increased as compared to untreated DBMSCs (DB), but was unchanged as compared to DBMSC migrating in response to 200 mM glucose [DB (To 200)], $p > 0.05$. **E** The effect of glucose on DBMSC invasion through endothelial cells by the xCELLigence system. At 10 h, the pretreatment with 200 mM glucose for 72 h [200 (Pre)] significantly increased DBMSC invasion as compared to untreated DBMSCs and DBMSC cultured with 200 mM glucose while the addition of 200 mM glucose [200 (in)] during the invasion experiment had no significant effect on DBMSC invasion as compared to untreated DBMSCs. Each experiment was performed in triplicate and repeated with five independent DBMSC (passage 3) preparations. * $p < 0.05$. Bars represent standard errors

3 Results

3.1 Glucose effect on DBMSC proliferation

DBMSCs were isolated using our established published method [1, 14–16] to assess the effect of glucose on their proliferation using the xCELLigence system. At 24 h, and as compared to untreated DBMSCs, the proliferation of DBMSCs unchanged at 25 mM glucose ($p > 0.05$), significantly increased at 50 and 200 mM glucose ($p < 0.05$), and was significantly reduced at 400 mM glucose, $p < 0.05$ (Fig. 1D). At 48 h, and as compared to untreated DBMSCs, the proliferation of DBMSCs unchanged at 50 mM glucose ($p > 0.05$), significantly increased at 200 mM glucose ($p < 0.05$), and significantly reduced at 25 and 400 mM glucose, $p < 0.05$ (Fig. 1E). Finally, at 72 h, and as compared to untreated DBMSCs, the proliferation of DBMSCs, significantly increased at 200 mM glucose ($p < 0.05$) but was significantly reduced at 25, 50

and 400 mM glucose, $p < 0.05$ (Fig. 1F). The viability of DBMSC treated with glucose (25–200 mM) for 72 h was $>90\%$ as determined by Trypan Blue exclusion. At 96 h, treatment with glucose (25–200 mM) reduced the viability of DBMSCs ($< 50\%$) while the treatment with 400 mM glucose reduced the viability of DBMSCs ($< 50\%$) at all examined culture times (24–72 h) (supplementary Fig. 1). Based on the results obtained above, the exposure time of 72 h and glucose at concentration of glucose (200 mM) was selected to evaluate the effect of glucose on DBMSC functions.

3.2 Reversibility of glucose effect on DBMSC proliferation

To evaluate the reversibility of the glucose effect on DBMSC proliferation, DBMSCs were initially cultured with 200 mM glucose for 72 h and their proliferation was then determined using the xCELLigence system. As

compared to untreated DBMSCs and DBMSC-treated with 200 mM glucose during the proliferation experiment [200 (pre)], the proliferation of DBMSC pretreated with 200 mM glucose [200 (pre)] significantly reduced ($p < 0.05$) at all examined culture times (24–72 h), (Fig. 1G–I). These results show the effect of glucose on DBMSC proliferation is reversible.

3.3 Glucose effect on DBMSC adhesion

To study the effects of glucose on the adhesion of DBMSCs, DBMSCs were cultured with 200 mM glucose and their adhesion was then determined using the xCELLigence system. At 2 h, and as compared to untreated DBMSCs, the adhesion of DBMSCs unchanged after treatment with 200 mM glucose, $p > 0.05$ (Fig. 2).

We also evaluated the reversibility of glucose effects on DBMSC adhesion. DBMSCs were initially cultured with 200 mM glucose for 72 h and their adhesion was then determined using the xCELLigence system. After 2 h, and as compared to untreated DBMSCs, and DBMSC-treated with 200 mM glucose during the experiment [200 (I)], the adhesion of DBMSCs pretreated with 200 mM glucose [200 (pre)] significantly increased, $p < 0.05$ (Fig. 2).

3.4 Glucose effect on DBMSC migration

To further study the effect of glucose on DBMSC functions, the migration of DBMSCs was monitored using the xCELLigence system. At 24 h and as compared to untreated DBMSCs (DB), the migration of DBMSCs in response to 200 mM glucose [DB (To 200)] significantly increased, $p < 0.05$ (Fig. 3D).

We also evaluated the reversibility of glucose effects on DBMSC migration, DBMSCs were initially cultured with 200 mM glucose for 72 h and their migration was then

determined using the xCELLigence system. The migration of DBMSC-pretreated with 200 mM glucose for 72 h in response to 200 mM glucose [Pre-DB (To 200)] significantly increased ($p < 0.05$) as compared to untreated DBMSCs (DB) but unchanged as compared to DBMSCs-migrated in response to 200 mM glucose [DB (To 200)], $p > 0.05$ (Fig. 3D). These results show the effect of glucose on DBMSC migration is irreversible.

3.5 Glucose effect on DBMSC invasion

We also evaluated the effect of glucose on DBMSC invasion through endothelial cells using the xCELLigence Real-Time Cell Analyser. In the xCELLigence Real-Time system, increased invasion is defined as an increase in the cell index due to the infiltration of HUVEC monolayer by DBMSCs and this therefore causing detachment of HUVEC while the increased of DBMSC adhesion is reflected by the increased of cell index defining the increase in cell invasion. At 10 h and as compared to untreated DBMSCs and DBMSCs cultured with 200 mM glucose, the pre-treatment of DBMSCs with 200 mM glucose for 72 h [200 (Pre)] significantly increased DBMSC invasion, $p < 0.05$ while the addition of 200 mM glucose [200 (in)] during the course of invasion experiment had no significant effect on DBMSC invasion, $p > 0.05$ (Fig. 3E).

3.6 Glucose increases DBMSC expression of adhesion and anti-inflammatory markers

To evaluate the modulatory effects of glucose on DBMSC functions, a variety of immune proteins important in DBMSC functions were studied by flow cytometry and expression recorded as median fluorescence intensity. As compared to untreated DBMSCs, after incubation with

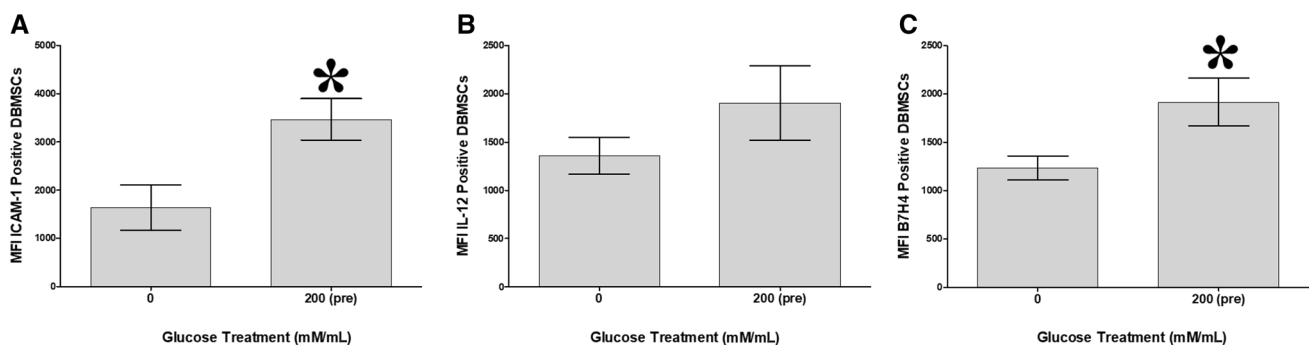


Fig. 4 Flow cytometric analysis of DBMSC expression of immune markers. A–C The treatment with 200 mM glucose significantly increased the DBMSCs [200 (pre)] expression of ICAM-1, had no significant effect on IL-12 expression, $p > 0.05$, and significantly

increased the expression of B7H4, as compared untreated DBMSCs. Each experiment was performed in triplicate and repeated with five independent DBMSC (passage 3) preparations. * $p < 0.05$. Bars represent standard errors

Table 1 Glucose effects on DBMSC expression of oxidative genes with survival, anti-apoptotic, proliferation, and migration properties. DBMSCs were untreated (DBMSC) or treated with 200 mM glucose (TDBMSC) for 72 h

#	Gene symbol	Gene full name	DBMSC Mean $\Delta\Delta^{-2}$ values	TDBMSC Mean $\Delta\Delta^{-2}$ values	Fold change (TDBMSC vs. DBMSC) $p < 0.05$	Biological properties
1	GPX2	Glutathione peroxidase 2	1	5	1 fold	↑ 1. Survival property
2	GPX3	Glutathione peroxidase 3	1	16	16 fold	↑ 2. Anti-apoptotic property
3	GPX4	Glutathione peroxidase 4	1	60	60 fold	↑ 3. Migration property
4	GPX7	Glutathione peroxidase 7	1	4	4 fold	↑ 4. Invasion property
5	PRDX1	Peroxiredoxin 1	1	1.5	1.5 fold	↑ 1. Survival property
6	PRDX4	Peroxiredoxin 4	1	2.52	2.52 fold	↑ 2. Migration property
7	PRDX5	Peroxiredoxin 5	1	2.63	2.63 fold	↑ 3. Invasion property
8	PRDX6	Peroxiredoxin 6	1	40	40 fold	↑
9	HMOX1	Heme oxygenase- 1	1	3.18	3.18 fold	↑ 1. Survival property 2. Migration property
10	ALB	Albumin	1	3	3 fold	↑ Survival property
11	OXR1	Oxidation resistance 1	1	12	12 fold	↑
12	KRT1	Keratin 1	1	2618	2618 fold	↑
13	NQO1	NAD(P)H dehydrogenase, quinone 1	1	21	21 fold	↑
14	MB	Myoglobin	1	707	707 fold	↑
15	STK25	Serine/threonine kinase 25	1	77	77 fold	↑ 1. Anti-apoptotic property 2. Migration property
16	ALOX12	Arachidonate 12-lipoxygenase	1	519	519 fold	↑ 1. Proliferation property 2. Migration property 3. Invasion property
17	TFII-I (GTF2I)	General transcription factor 2I	1	60	60 fold	↑ Proliferation property
18	FOXM1	Forkhead box M1	1	34	34 fold	↑
19	BNIP3	BCL2/adenovirus E1B 19 kDa interacting protein 3	1	49	49 fold	↑ Migration property

200 mM glucose for 72 h [200 (pre)], DBMSC expression of ICAM-1 and B7H4, significantly increased $p < 0.05$ (Fig. 4), while the expression of IL-12 by DBMSCs did not significantly change, $p > 0.05$ (Fig. 4B).

3.7 Glucose modulated the expression of genes important in DBMSC functions

The expression of oxidative stress-associated genes by DBMSCs was studied after culturing DBMSCs with 200 mM glucose for 72 h, and then analysed and assessed using the RT-PCR. Results show that glucose modulated DBMSC expression of variety of genes associated with many cellular functions as compared to untreated DBMSC as shown in Tables 1, 2, 3, 4 and 5.

4 Discussion

Recently, we reported the therapeutic potential of DBMSCs to treat inflammatory diseases, such as atherosclerosis and cancer [14–16, 19]. Diabetes is another inflammatory diseases [8] where high levels of glucose (an oxidative stress mediator) cause cellular and tissue damage [20–23]. Therefore, for an effective application of DBMSCs in diabetes, it is important for DBMSCs maintain their normal reparative properties when exposed to high levels of glucose. Here, we studied the functional and phenotypic changes of DBMSCs in response to glucose.

First, we examined the effect of different glucose concentrations on the survival of DBMSCs. We report that

Table 2 Glucose effects on DBMSC expression of oxidative genes with pro-oxidant and antioxidant properties. DBMSCs were untreated (DBMSC) or treated with 200 mM glucose (TDBMSC) for 72 h

#	Gene symbol	Gene full name	DBMSC Mean $\Delta\Delta^{-2}$ values	TDBMSC Mean $\Delta\Delta^{-2}$ values	Fold change (TDBMSC vs. DBMSC) $p < 0.05$	Biological properties
1	NOX2 (CYBB)	Cytochrome b-245, β polypeptide	1	11.62	11.62 fold	↑ Pro-oxidant property
2	NOX4	NADPH oxidase 4	1	9.89	> 9 fold	↑
3	NOX5	NADPH oxidase 5	1	14	14 fold	↑
4	DUOX1	Dual oxidase 1	1	2	2 fold	↑
5	DUOX2	Dual oxidase 2	1	52	52 fold	↑
6	NCF1	Neutrophil cytosolic factor 1	1	6.59	6.59 fold	↑
7	NCF2	Neutrophil cytosolic factor 2	1	50	50 fold	↑
8	ALOX12	Arachidonate 12-lipoxygenase	1	519	519 fold	↑
9	AOX1	Aldehyde oxidase 1	1	34	34 fold	↑
10	GPX1	Glutathione peroxidase 1	1	0.10	10 fold	↓ Anti-oxidant property
11	GPX5	Glutathione peroxidase 5	1	13	13 fold	↓
12	GPX6	Glutathione peroxidase 6	1	0.02	50 fold	↓
13	PRDX2	Peroxiredoxin 2	1	0.02	50 fold	↓
14	PRDX3	Peroxiredoxin 3	1	0.39	> 2.50 fold	↓
15	CAT	Catalase	1	0.56	> 1.70 fold	↓
16	SOD1	Superoxide dismutase 1	1	0.016	> 62 fold	↓
17	SOD2	Superoxide dismutase 2	1	0.052	> 19 fold	↓
18	TTN	Titin	1	0.20	5 fold	↓

DBMSCs survive in high levels of 200 mM glucose even when cultured for long periods. DBMSCs also showed increased proliferation potential at high levels of glucose (Fig. 1D–F), via a reversible mechanism (Fig. 1G–I). This contrasts with our previous finding in which MSCs from the chorionic villi of human placenta (pMSCs) show a reduction in their proliferation under the effect of glucose [18]. This may reflect DBMSC adaption to the elevated oxidative stress levels in normal pregnancy, as a result of their vascular microenvironment where they are directly or indirectly exposed to factors in maternal pregnant blood, which contains high level of oxidative stress mediators [24]. On the other hand, pMSCs in the chorionic vascular niche, are exposed to the fetal circulation, which contains relatively reduced levels of oxidative stress throughout normal pregnancy [13, 25].

Glucose also induced DBMSC expression of genes associated with survival, anti-apoptotic [26–37], and proliferation [38–40] as shown in Table 1. These molecules may prevent the damaging effects of glucose on DBMSCs. However, this needs further investigation to confirm the protective roles of these molecules in the survival and proliferation of DBMSCs from glucose. In this study, preconditioning of DBMSCs with glucose enhanced their adhesion (Fig. 2) possibly via ICAM-1 (Fig. 4A). This is

consistent with our previous study which showed that DBMSCs preconditioned with H_2O_2 showed increased adhesion [14]. Other studies also support our finding, where preconditioning of hematopoietic stem cells with H_2O_2 also increased their adhesion *in vitro* and *in vivo* [41]. Adhesion is the first important biological process required for a successful stem cell engraftment [42, 43]. Migration and invasion of MSCs are other important biological processes that occur during MSC engraftment in a disease environment with high level of oxidative stress mediators [42, 43]. We found that DBMSCs preconditioned with glucose improved their migration (Fig. 3D). This effect is similar to the effect of H_2O_2 on the migration of DBMSCs [14], MSCs from the chorionic villi [44] and bone marrow [45]. DBMSCs preconditioned with glucose also improved their invasion (Fig. 3E) via a mechanism that may involve the induction of a number of genes known for their migratory [26–29, 31, 36, 46–51] and invasive properties [26–28, 47, 48], Table 1. These results demonstrate that the engraftment properties of DBMSCs can be improved by glucose pretreatment, possibly via these genes. Thus, preconditioning DBMSCs could be valuable component of cell-based therapies that must act in high oxidative stress environments. However, a future mechanistic study is necessary to confirm this further.

Table 3 Glucose increased DBMSC expression of genes with antioxidant, anti-inflammatory, anti-chemoattractant, and antimicrobial properties. DBMSCs were untreated (DBMSC) or treated with 200 mM glucose (TDBMSC) for 72 h

#	Gene symbol	Gene full name	DBMSC Mean $\Delta\Delta^{-2}$ values	TDBMSC Mean $\Delta\Delta^{-2}$ values	Fold change (TDBMSC vs. DBMSC) $p < 0.05$	Biological properties
1	GPX2	Glutathione peroxidase 2	1	5	1 fold	↑ Anti-oxidant property
2	GPX3	Glutathione peroxidase 3	1	16	16 fold	↑ 2. Anti-inflammatory property
3	GPX4	Glutathione peroxidase 4	1	60	60 fold	↑
4	GPX7	Glutathione peroxidase 7	1	4	4 fold	↑
5	UCP2	Uncoupling protein 2	1	7	7 fold	↑
6	SEPP1	Selenoprotein P, plasma, 1	1	10	10 fold	↑
7	PRDX1	Peroxiredoxin 1	1	1.5	1.5 fold	↑ Anti-oxidant property
8	PRDX4	Peroxiredoxin 4	1	2.52	2.52 fold	↑
9	PRDX5	Peroxiredoxin 5	1	2.63	2.63 fold	↑
10	PRDX6	Peroxiredoxin 6	1	40	40 fold	↑
11	TPO	Thyroid peroxidase	1	2.48	2.48 fold	↑
12	TFII-I (GTF2I)	General transcription factor 2I	1	60	60 fold	↑
13	BNIP3	BCL2/adenovirus E1B 19 kDa interacting protein 3	1	49	49 fold	↑
14	PNKP	Polynucleotide kinase 3'-phosphatase	1	2.75	2.75 fold	↑
15	SOD3	Superoxide dismutase 3	1	4.45	4.45 fold	↑ Anti-oxidant property Anti-inflammatory property 3. Anti-chemoattractant property
16	HMOX1	Heme oxygenase- 1	1	3.18	3.18 fold	↑ Anti-inflammatory property
17	MT3	Metallothionein 3	1	124	124 fold	↑
18	GSR	Glutathione reductase	1	2.55	2.55 fold	↑
19	DUSP1	Dual specificity phosphatase 1	1	2	2 fold	↑
20	KRT1	Keratin 1	1	2618	2618 fold	↑
21	NQO1	NAD(P)H dehydrogenase, quinone 1	1	21	21 fold	↑
22	SIRT2	Sirtuin 2	1	2.99	2.99 fold	↑
23	SFTPD	Surfactant protein D	1	114	114 fold	↑ Anti-inflammatory property
24	MBL2	Mannose-binding lectin (protein C) 2	1	6.34	6.34 fold	↑ 2. Antimicrobial property

In the pancreatic beta islets, the pro-oxidant enzymes (i.e. NOX1-5 and DUOX1-2) increase the production of the reactive oxygen specie (ROS) superoxide, which induces insulin secretion [52–56]. The excessive accumulation of ROS causes beta cell damage, which can be prevented by the antioxidant enzymes (i.e. GPX, CAT and SOD), which act as ROS scavengers, and therefore inhibit insulin secretion [52–56]. In this study, glucose induced and reduced DBMSC expression of genes with pro-oxidant [39, 57, 58] and anti-oxidant properties, respectively [59], Table 2. Thus, indicating that glucose may direct DBMSCs to activate pathways associated with insulin secretion. This postulate is supported by the finding that glucose also induced DBMSC expression of albumin and NOS2, which are associated with insulin secretion [32, 60]. In addition,

glucose also reduced DBMSC expression of PXDN, a molecule that triggers diabetes, Table 4 [61].

Generally, a basal level of ROS is required to stimulate basic cellular biological activities (i.e. proliferation, migration, and invasion). ROS is also required for insulin secretion by beta cells. As discussed above, the high level of ROS damages tissue, and consequently this is avoided by the antioxidant enzymes which are produced to scavenge ROS [62]. Glucose simultaneously induced DBMSC expression of both pro-oxidant (Table 4) and anti-oxidant genes [40, 50, 63–66], Table 4. Therefore, DBMSCs may respond to glucose induction of ROS by generating antioxidants to prevent cellular damage and also to regulate insulin secretion probably by inducing the expression of UCP2 (Table 4), which has anti-insulin secretion activity [63].

Table 4 Glucose effects on DBMSC expression of oxidative genes. DBMSCs were untreated (DBMSC) or treated with 200 mM glucose (TDBMSC) for 72 h

#	Gene symbol	Gene full name	DBMSC Mean $\Delta\Delta^{-2}$ values	TDBMSC Mean $\Delta\Delta^{-2}$ values	Fold change (TDBMSC vs. DBMSC) $p < 0.05$	Biological properties
1	CYGB	Cytoglobin	1	0.56	> 1.70 fold ↓	Cytoprotective property
2	TXNRD2	Thioredoxin reductase 2	1	18	18 fold ↑	Anti-growth property
3	NOS2	Nitric oxide synthase 2	1	15.81	15.81 fold ↑	Inflammatory property
4	MGST3	Microsomal glutathione S-transferase 3	1	0.34	> 2.9 fold ↓	
5	COX1	Cyclooxygenase 1	1	2	2 fold ↑	
6	COX2	Cyclooxygenase 2	1	0.0077	> 129 fold ↓	
7	NOX4	NADPH oxidase 4	1	9.89	> 9 fold ↑	
8	NOX5	NADPH oxidase 5	1	14	14 fold ↑	
9	GSTZ1	Glutathione transferase zeta 1	1	0.0002	5000 fold ↓	Anti-inflammatory property
10	GSTP1	Glutathione S-transferase pi 1	1	0.06	> 16 ↓	Anti-apoptotic Property 2. Anti-inflammatory property
11	UCP2	Uncoupling protein 2	1	7	7 fold ↑	Inhibits Insulin Secretion
12	PXDN	Peroxidasin homolog	1	0.24	> 4 fold ↓	Triggers Diabetes
13	BNIP3	BCL2/adenovirus E1B 19 kDa interacting protein 3	1	49	49 fold ↑	Pro-apoptotic property
14	PRNP	Prion protein	1	0.05	20 fold ↓	Anti-inflammatory property
15	HSP70-1A	Heat shock 70 kDa protein 1A	1	0.05	20 fold ↓	
16	PDLIM1	PDZ and LIM domain 1	1	0.01	100 fold ↓	
17	TTN	Thioredoxin	1	0.10	10 fold ↓	Chemoattractant property
18	SOD1	Superoxide dismutase 1	1	0.016	> 62 fold ↓	Antioxidant property
19	SOD2	Superoxide dismutase 2	1	0.052	> 19 fold ↓	Anti-inflammatory property 3. Anti-chemoattractant property
20	LPO	Lactoperoxidase	1	0.029	> 34 fold ↓	Antimicrobial property 2. Oxidative property

In diabetes, the oxidative stress mediators generated by the high level of glucose, stimulate the recruitment of immune cells to the site of tissue injury, and this in return will intensify tissue damage [67]. One of the therapeutic strategies, is to reduce the recruitment of immune cells to the injured tissue. In this study, glucose reduced DBMSCs expression of thioredoxin (Table 4), an oxidative stress molecule that increases the recruitment of immune cells [67]. Glucose also increased the anti-inflammatory properties of DBMSCs by increasing their expression of anti-inflammatory genes [26, 31, 34, 35, 63, 64, 68–74] (Table 3), and also by reducing their expression of pro-inflammatory genes including MGST3 and COX2 [75–77]. This finding is important, because these anti-inflammatory molecules reduce the recruitment of immune cells [70]. These results indicate that DBMSCs may function as an anti-chemoattractant agent to reduce the recruitment of immune cells to the injured tissues in inflammatory diseases. The property of DBMSCs to ameliorate

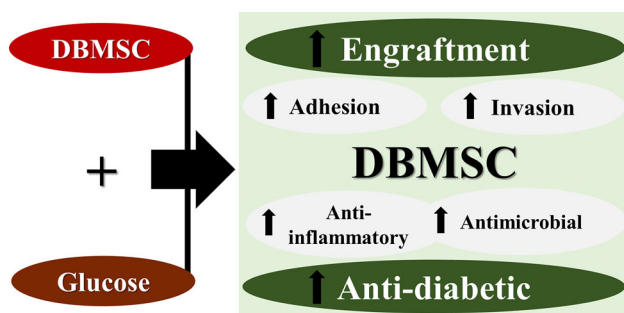
inflammation is further confirmed by their enhanced expression of B7-H4 in response to glucose (Fig. 4). B7-H4 is a protein that inhibits T-cell proliferation [78], suggesting that DBMSCs may inhibit T cell proliferation, and thus reduce inflammation.

High levels of glucose also causes immune dysfunction that results in a reduction in the antimicrobial activity of the immune cells in diabetic patients, and therefore these patients are at a higher risk to bacterial infection [79]. In this study, glucose induced DBMSC expression of antibacterial genes, [71, 73], Table 3. Collectively, these results suggest that DBMSCs may have the potential to treat inflammatory diseases, such as diabetes, by ameliorating inflammation and also by preventing infection associated with hyperglycaemia.

This is the first study to show the beneficial effects of glucose on DBMSC functions. Preconditioning of DBMSCs with glucose may increase their therapeutic potential by enhancing their engraftment efficiency

Table 5 Glucose effects on DBMSC expression of oxidative genes. DBMSCs were untreated (DBMSC) or treated with 200 mM glucose (TDBMSC) for 72 h

#	Gene symbol	Gene full name	DBMSC Mean $\Delta\Delta^{-2}$ values	TDBMSC Mean $\Delta\Delta^{-2}$ values	Fold change (TDBMSC vs. DBMSC)
1	EPX	Eosinophil peroxidase	1	1.09	Fold Change is not statistically significant, $p > 0.05$
2	APOE	Apolipoprotein E	1	1.26	
3	TXNRD1	Thioredoxin reductase 1	1	0.74	
4	EPHX2	Epoxide hydrolase 2, cytoplasmic	1	0.81	
5	APOE	Apolipoprotein E	1	1.26	
6	GCLM	Glutamate-cysteine ligase, modifier subunit	1	0.88	
7	GPX1	Glutathione peroxidase 1	1	0.10	
8	FTH1	Ferritin, heavy polypeptide 1	1	0.62	
9	EPX	Eosinophil peroxidase	1	1.09	
10	MSRA	Methionine sulfoxide reductase A	1	0.71	
11	OXSRI	Oxidative-stress responsive 1	1	0.88	
12	RNF7	Ring finger protein 7	1	0.61	
13	SCARA3	Scavenger receptor class A, member 3	1	0.79	
14	SQSTM1	Sequestosome 1	1	0.65	
15	NUDT1	Nudix (nucleoside diphosphate linked moiety X)-type motif 1	1	0.37	
16	SRXN1	Sulfiredoxin	1	1.72	
17	PREX1	Phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 1	1	1.59	
18	CCS	Copper chaperone for superoxide dismutase	1	0.65	
19	DHCR24	24-dehydrocholesterol reductase	1	1.40	
20	GCLC	Glutamate-cysteine ligase, catalytic subunit	1	1.90	
21	GSS	Glutathione synthetase	1	1.89	
22	MPV17	MpV17 mitochondrial inner membrane protein	1	1.68	
23	ATOX1	ATX1 antioxidant protein 1 homolog (yeast)	1	1.86	
24	CCL5	Chemokine (C–C motif) ligand 5	1	0.67	
25	MPO	Myeloperoxidase	1	1.58	

**Fig. 5** Illustration showing the treatment of DBMSCs with glucose increases their functional (adhesion, invasion and engraftment) and phenotypic (anti-inflammatory, anti-microbial and anti-diabetic) properties

(Fig. 5). In addition, the induction of ROS in DBMSCs by glucose may program these cells into insulin producing cells with ability to counteract inflammation and infection

associated with diabetes (Fig. 5). However, future *in vitro* and *in vivo* studies are essential to investigate the findings of this study further.

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Author Contributions MHA and TK proposed and supervised the project. MHA and TK designed the experiments. YSB, MAR, MAA, and EB performed the experiments. MHA, YSB and TK analysed the data. YSB, TK and MHA wrote the manuscript. MHA, YSB, TK, MLA, BK, SM and FMA, contributed to data analysis and interpretation of results. All authors reviewed the manuscript.

Compliance with ethical standards

Conflict of interest No competing financial interests exist. The authors declare that there is no conflict of interests regarding the publication of this paper.

Ethical statement The institutional review board (IRB) at King Abdullah International Medical Research Centre (KAIMRC), Saudi Arabia approved this study. Samples (i.e. placentae and umbilical cords) were obtained from uncomplicated human pregnancies (38–40 gestational weeks) following informed patient consent.

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