PGC-1α attenuates the oxidative stress-induced impaired osteogenesis and angiogenesis regulation effects of mesenchymal stem cells in the presence of diabetic serum

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A B S T R A C T

Oxidative stress is believed to induce dysfunction of the bone remodeling process and be associated with progressive loss of bone mass. The peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α) is a master controller during mitochondrial biogenesis and the antioxidant response. We postulated that PGC-1α could function as a cyto-protective effector in mesenchymal stem cells (MSCs) under oxidative stress conditions. In this study, diabetic serum was firstly used to treat MSCs to induce oxidative damage. The anti-oxidative protective effects of PGC-1α overexpression on MSCs, as well as MSCs' osteogenesis and angiogenic regulation effects were investigated *in vitro*. Results showed that diabetic conditions induced significantly increase of intracellular oxidative damage and mitochondrial permeability transition pore (mPTP) opening activity, decrease of cellular viability, and osteogenic differentiation and pro-angiogenic regulation effects of MSCs. However, the diabetic conditions induced oxidative impair on MSCs were significantly alleviated via PGC-1α overexpression under diabetic conditions. Taken together, this study indicates the anti-oxidative treatment potential of PGC-1α regulation as a promising strategy to promote coupling pro-osteogenesis and pro-angiogenesis effects of MSCs.

1. Introduction

Recent studies have proved that oxidative stress induced by overproduction of reactive oxygen species (ROS) is associated with progressive loss of bone mass [1,2]. Numerous pathological conditions including aging, inflammation, and diabetes mellitus could cause a chronic ROS overproduction [3,4]. For example, hyperglycemia in diabetes could cause significantly increase level of ROS in both mitochondria and cytoplasm [5]. The increased ROS leads to imbalance of cellular oxidant/antioxidant system and cellular damage, such as increase of osteogenic cells apoptosis and decrease of osteoblast number [2,6]. In stem cells, a high level of ROS was also proved to inhibit survival, proliferation, and differentiation potency of the naive stem cells towards osteogenic lineage via regulating several signaling cascades [7,8]. Recently, studies have focused on the regulation effects of antioxidant on osteogenesis under oxidative stress conditions. Administration of antioxidants such as *N*-acetyl-ʟ-cysteine (NAC), graft chitosan

derivatives and Vitamin D3 significantly decreased the intracellular ROS production of MSCs, attenuate the oxidative damage of MSCs under oxidative stress environment, and contribute to promoted cellular functions and osteogenesis [9,10]. What's more, using gene transfection or siRNA interference to regulate the expression levels of certain genes and redox pathways were also proved to reduce the endogenous ROS levels and favors osteogenic differentiation of MSC [8].

The peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α), encoded by PPARGC1A, is described as a master controller of mitochondrial biogenesis and oxidative metabolism via regulation of several thermogenic genes expression [11]. Dysfunction of PGC-1a was linked to the pathogenesis of insulin resistance in type 2 diabetes [12]. What's more important, it is also indicated that PGC-1α plays a vital role in mediating the defense against ROS [3] and preventing cellular aging. Recently papers suggested that deletion of PGC-1a in mesenchymal cell-specific promoted bone loss, yet induction of PGC-1a promotes bone formation [13]. However, relatively little was known about the

regulation effects of PGC-1a on MSC's osteogenic differentiation under oxidative stress conditions as well as the underlying mechanism. Besides, well-documented literatures suggested that in nature, coupled angiogenesis and osteogenesis regulation via the cross-talk between osteoblastic cells and angiogenic ECs play critical roles during bone formation and remodeling process [14,15]. Whether PGC-1a could regulation the angiogenic effect of ECs via MSCs under oxidative stress condition is still unknown.

In this study, diabetic serum was firstly used to treat MSCs to created diabetes-induced ROS overproduction. Anti-oxidative osteogenesis regulation effects of PGC-1a-overexpression on MSCs were investigated *in vitro*. After that, the paracrine effect of PGC-1a over-expressed MSCs on ECs angiogenesis was further investigated as well.

2. Materials and methods

2.1. Materials

Human umbilical vein endothelial cells |(HUVEC) and human bone marrow derived MSCs were purchased from Saiye Co. (China). For over-expression of human PGC-1α in MSCs cells, the lentiviral vector encoding the MOCK or FLAG-tagged human PGC-1α (PGC-1α) under the control of the CMV promoter were constructed and purchased from Hanbio (China). Osteogenic differentiation medium for human MSCs were bought from (HUXMA-90021, Cyagen, China).

2.2. Animals

This study was carried out in strict in accordance with the National Institutes of Health Guidelines for the Use of Laboratory Animals. All methods were approved by the Animal Care Regulations (ACR) Committee of Beijing Fangshan District Liangxiang Hospital (Permit Number: 2018-dw0803). The diabetic model was constructed with similar method published previously [9,16]. Briefly, diabetes was induced in twelve New Zealand rabbits (Male, 6 months age, 3.5–3.75 kg) by intraperitoneal administration of monohydrated alloxan (Sigma-aldrich, St. Louis, MO, USA) at 150 mg/kg in 5% glucose solution to prevent hypoglycemia. The rabbits with serum glucose levels $\geq$ 16.67 mmol/L at both 3 days and 3 weeks after injection were considered diabetic. Finally, 9 Diabetic rabbits were successfully induced (from a total of 12). Serum of normal and diabetic rabbits were acquired respectively and serum parameters were characterized (Table S2).

2.3. Cell culture

HUVEC were cultivated in endothelial cell growth medium (HUVEC-90011, Cyagen, China) with Endothelial Cell Growth Supplement (ECGS). Human bone marrow derived MSCs were cultured and amplified in complete DMEM-F12 media (GIBCO, USA) supplemented with 10% fetal bovine serum (GIBCO, USA), 1% penicillin/streptomycin. All cells were maintained in a humidified incubator at 37 °C under a 5% CO_2 atmosphere. The cells were passaged according to the manufacturer's instructions as they reached around 95% confluence.

2.4. Role of PGC-1α in MSC under oxidative stress condition

To investigate the involvement of PGC-1α in diabetic serum (DS) treated MSC, the MSCs were treated with complete DMEM-F12 media (GIBCO, USA) supplemented with 10% DS, 1% penicillin/streptomycin. The expression levels of PGC-1α mRNA and protein contents at 0, 6, 12 and 24 h (h) time points were measured by RT-PCR and western blotting respectively. To further evaluate the expression levels of PGC-1α protein under antioxidant treatment, the MSCs were treated with normal or diabetic serum for 24 h, with or without adding of NAC (5 mmol/L), and the relative mRNA expression and protein contents of PGC-1α were measured by RT-PCR and western blotting respectively as well.

2.5. Transfection of MSCs

Transduction of MSCs was performed in the 6-well plate (Corning). Lentivirus supernatants in 1 mL culture medium were added to 1×10^6 target cells at m.o.i.of 10 for 4 h and followed by adding 1 mL of culture medium. After transfection for 24 h, the culture medium was replaced with fresh medium for further experiments. Expression of Flag and human PGC-1a expression were confirmed by western blotting.

2.6. Experimental design

The normal MSCs or PGC-1α overexpressed MSCs were firstly seeded in 24-well plates at 4×10^4 per well and cultured for 12 h for fully cell adhesion. After that, the medium was replaced and the normal MSCs or PGC-1α overexpressed MSCs were cultured with certain condition as following: 1) Control group, normal MSCs were treated with normal rabbit serum; 2) DS group, normal MSCs were treated with diabetic rabbit serum; 3) PGC-1α, the PGC-1α overexpressed MSCs were treated with normal rabbit serum; 4) PGC-1α+ DS group, the PGC-1α overexpressed MSCs were treated with diabetic rabbit serum (DS).

2.7. Cell viability assessment

To measure the cellular viability of MSCs from different groups at 0, 12 and 24 h time points after treatment, 3-(4,5-dimethylithiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was performed as previously published and optical density (OD) at 490 nm was measured on a spectrophotometric microplate reader (Bio-Rad 680, USA).

2.8. Mitochondrial permeability transition pore (mPTP) opening analysis

After culture for 24 h, the opening of mPTPs was assessed by flow cytometry and the MitoProbe™ Transition Pore Assay Kit was used according to the manufacturer's instructions (Thermo Fisher Scientific, Inc., USA). The samples were analyzed using a flow cytometer (Beckman Coulter, Brea, CA, USA) with 488-nm excitation and 517-nm emission wavelengths.

2.9. Evaluation of cellular oxidative status and SOD gene

To evaluate the cellular oxidative status of MSCs, the intracellular reactive oxygen species levels, Malondialdehyde (MDA) and superoxide dismutase (SOD) activity were measured after 24 h of incubation. Briefly, the levels of intracellular total ROS and H_2O_2 were analyzed using flow cytometry. For cytosolic ROS level evaluation, the cells were treated with 10 μM FDCFH-DA for 15 min at 37 °C and the fluorescence intensities were quantified using a flow cytometer (Beckman Coulter, Brea, CA, USA). For H_2O_2 level evaluation, the cells were incubated with DHE (5 μM) for 20 min under the dark condition and the fluorescence intensities were quantified using a flow cytometer (Beckman Coulter, Brea, CA, USA). The Lipid Peroxidation MDA Assay Kit (Beyotime, China) for Malondialdehyde (MDA) assay and the Cu/Zn-SOD and Mn-SOD Assay Kit (Beyotime, China) for superoxide dismutase (SOD) activity detection were performed according to the manufacturer's instructions.

Meanwhile, the mRNA levels of superoxide dismutase (SOD) genes, including SOD1, SOD2, SOD3, were measured by RT-PCR. The primers for the targeted genes tests were presented in the Table S1 in Supporting Information.Doc. β-Actin was used as the internal control and each experiment was repeated for 3 times.

2.10. qRT-PCR for assessment of mRNA level

To quantify the transcript levels of osteogenesis-related genes, MSCs were cultured in osteogenic differentiation medium and FBS was completely replaced with normal or diabetic serum. Total mRNA was

extracted at 7th day and 14th day after culture, and gene expression was evaluated using TB Green Premix Ex Taq II (Tli RNaseH Plus) on the ABI-7500 PCR System. The primers for the targeted genes tests were presented in the Table S1 in Supporting Information.Doc. β-Actin was used as the internal control and each experiment was repeated for 3 times.

2.11. Alkaline phosphatase (AKP) activity evaluation

To evaluate the pro-osteogenesis effect of PGC-1α overexpression on MSCs under oxidative stress, the ALP activity of MSCs was analyzed after culture for 7 days in osteogenic differentiation medium. AKP activity of MSCs were evaluated via both ALP staining using a BCIP/NBT alkaline phosphatase staining kit and Alkaline Phosphatase Assay Kit (Beyotime, China).

2.12. Alizarin red staining (ARS) for mineralization assay

After culturing for 21 days in osteogenic differentiation medium, the ECM mineralization of MSCs were evaluated via Alizarin red staining and quantitative assay respectively.

2.13. Enzyme-linked immunosorbent assay (ELISA) for OCN and OPN

After cultured for 21 days in osteogenic differentiation medium, the concentration of secreted OCN and OPN were measured using a solid-phase sandwich human OCN and OPN ELISA kit (CUSABIO, China) following the manufacturer's instructions (n = 5).

2.14. Pro-angiogenic effect evaluation

The gene expression of angiogenic factors in MSCs at 7 days after treatment were measured via qRT-PCR assay method as mentioned above. To evaluate the effect of MSC-conditioned medium on HUVECs' capillary-like tube formation, the medium of four groups were removed, washed twice with FBS-free DMEM-F12 media and replaced with equal volume of normal medium: DMEM-F12 media (GIBCO, USA) supplemented with 10% FBS and 1% penicillin/streptomycin after culture for 7 days. After incubation for further 24 h, the conditioned media (CMs) were collected from each group. After that, HUVECs were seeded in growth factor reduced Matrigel (BD Bioscience; 100 μl per well) pre-coated 48-well plates at 2×10^4 cells per well in 250 μl conditioned medium from different groups. After being incubated for 6 h, the HUVECs were stained by fluorescein diacetate (FDA, 5 μmol/L) and visualized with IXplore Pro microscope system (OLYMPUS Corporation, Japan).

To further measure the paracrine effects of MSCs on immigration of HUVECs, the medium of four groups were firstly replaced with equal volume of FBS-free DMEM-F12 media after culture for 7 days [17]. The HUVECs were seeded into the Matrigel pre-coated upper transwell chamber with 8.0 μm pore polycarbonate membrane at a density of 1×10^4 cells per well. After culture for 24 h, migrated cells were fixed and stained. The migrated HUVECs were observed with IXplore Pro microscope system (OLYMPUS Corporation, Japan). Images of 3 random separate fields from each group were recorded (n = 5).

2.15. Statistical analysis

All data were present as means ± standard deviation (SD). The statistical analysis was carried out with OriginPro (8.0) via Student's t-test for single comparisons or the one-way ANOVA for multiple comparisons. The confidence levels were set as 95% and 99%.

3. Results

3.1. Diabetic serum downregulated PGC-1αexpression of MSCs

As shown in Fig. 1A and B, the results of both PGC-1α protein contents and mRNA expression levels showed that PGC-1α decreased significantly overtime past 6 h (P < 0.05 or 0.01c). However, even being treated with diabetic serum for 24 h, the decreased PGC-1α protein content and mRNA expression level could be remarkably restored via adding antioxidant NAC (Fig. 1C and D). These results indicated that PGC-1α expression in MSCs was downregulated under diabetic serum treatment condition, yet administration of antioxidant NAC could restore the expression level of PGC-1α.

3.2. Regulation effect of PGC-1α overexpression on cellular viability and mPTP

As shown in Fig. 2A, the expression of c-terminal Flag tagged PGC-1α was assessed with anti-Flag antibody. Compared with normal MSCs in TCPS group or MSCs in MOCK group, PGC-1α transfected MSCs (PGC-1α) were observed with the expression of Flag and the PGC-1a overexpression could sustain for at least 14 d after transfection (Fig. S1). The cell viability results showed that there were no significant differences among the four groups at 0 h (p > 0.05). However, compared with Control and PGC-1α groups, DS treatment significantly decrease the cellular viability of MSCs at 12 h and 24 h (p < 0.01). Over-expression of PGC-1 α could significantly increase (p < 0.01) the MSCs' viability at 12 h and 24 h (Fig. 2B). To investigate the status of MPTP activity, mitochondrial permeability transition pore (MPTP) opening was analyzed with flow cytometer. As shown in Fig. 2C, as compared with MSCs in the Control group, the MPTP opening activity was decreased after DS treatment for 24 h. However, PGC-1 α over-expression could attenuate the DS-induced mPTP opening in PGC-1 α+ DS group. All these results indicated that PGC-1 α over-expression alleviates DS-induced decreased cellular viability and opening of mPTP.

3.3. PGC-1α overexpression alleviates DS-mediated oxidative injury

As shown in Fig. 2D–E, fluorescent observation and flow cytometer results proved that both DHE and DCF fluorescent intensity in DS group were much higher than that in Control, PGC-1 α and PGC-1 α+ DS groups. In order to further evaluate the oxidative injury of MSCs, the Lipid Peroxidation MDA Assay and SOD activity detection were performed as well. As shown in Fig. 2H & I, diabetic serum significantly decreases the MDA and SOD activity of MSCs in DS group as compared with Control group (p < 0.01). Although SOD activity of PGC-1 α group (18.9 ± 2.23) is higher than Control group (18.4 ± 1.19), no significantly difference was observed. However, the MDA and SOD activity were remarkably restored via PGC-1α over-expression in PGC-1α + DS group (p < 0.05).

To preliminary investigate the potential underlying mechanism involved in the antioxidant protective effects of PGC-1α overexpression on MSCs under oxidative stress, the mRNA levels of different SOD genes were analyzed using RT-PCR. As shown in Fig. 2H, only mRNA expression level of SOD2 was significantly increased via PGC-1 α overexpression ($p < 0.05$). Compared with Control and PGC-1α groups, DS treatment significantly decrease the mRNA expression levels of SOD1, 2, 3 genes (p < 0.05 or p < 0.01). However, compared with both SOD1 and SOD3, only decrease of SOD2 mRNA expression level under DS treatment was significantly alleviated (#p < 0.05). These results confirmed that PGC-1α overexpression alleviates diabetic serum-induced oxidative stress of MSCs.
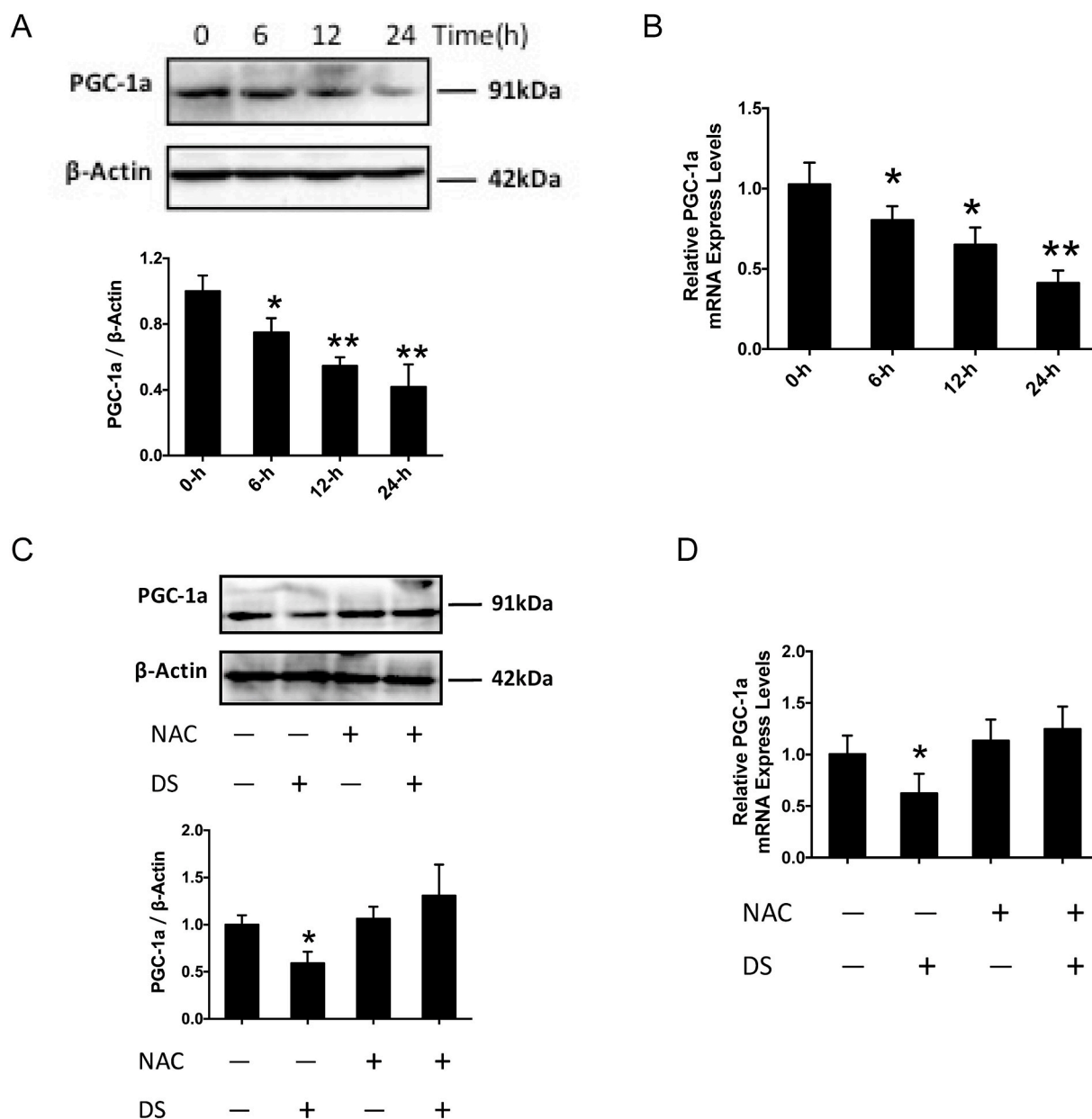
Fig. 1. Measurement of PGC-1α expression in MSC under oxidative stress condition. (A) Western blotting detection of PGC-1α protein contents and (B) RT-PCR quantitative assay of PGC-1α mRNA expression levels at 0, 6, 12 and 24 h time points post diabetic serum treatment, *P < 0.05 and **P < 0.01, vs. Group 0-h; (C) Western blotting detection of PGC-1α protein contents and (D) RT-PCR quantitative assay of PGC-1α mRNA expression levels of MSCs treated with normal or diabetic serum with or without adding of NAC (5 mmol/L) for 24 h; *P < 0.05 vs. control group NAC-/DS-.

3.4. PGC-1α over-expression contributed to increased osteogenic differentiation of MSCs under diabetic serum treatment

As shown in Fig. 3A, compared with MSCs in Control group, MSCs treated with diabetic serum in DS group were observed with significantly decreased Runx-2, Collagen 1, OCN and OPN at day 7th or 14th (p < 0.05 or 0.01). However, PGC-1α over-expression in PGC-1 α + DS group significantly increased the expression of these osteogenesis-relative genes compared to the DS group (p < 0.05 or 0.01).

ALP evaluation results showed that (Fig. 3B and D), compared to Control group, DS group was observed with decreased level of ALP activity (p < 0.01). However, compared to DS group, ALP activity in PGC-1 α+DS group (p < 0.05) was significantly increased. To further evaluate the osteogenic differentiation of MSCs in different groups, the ECM mineralization was analyzed with alizarin red staining and related quantitative analysis. As shown in Fig. 3C & E, compared to Control

group, DS group were observed with reduced positive regions and significantly decreased statistical ECM mineralization. However, the downregulation effect by DS treatment was attenuated via PGC-1α over-expression in PGC-1α + DS group. Similar to qRT-PCR results, compared with Control group, the secreted levels of OCN and OPN in MSCs were significantly decreased via diabetic serum treatment in DS group. Whereas PGC-1α over-expression could significantly increase OCN and OPN levels in PGC-1 α+DS. All these results provided strong evidence that diabetic serum-induced oxidative stress leads to impaired osteogenesis of MSCs, while PGC-1 α over-expression could alleviate the diabetic serum -mediated oxidative injury and contributed to increased osteogenic differentiation of MSCs under diabetic serum treatment.
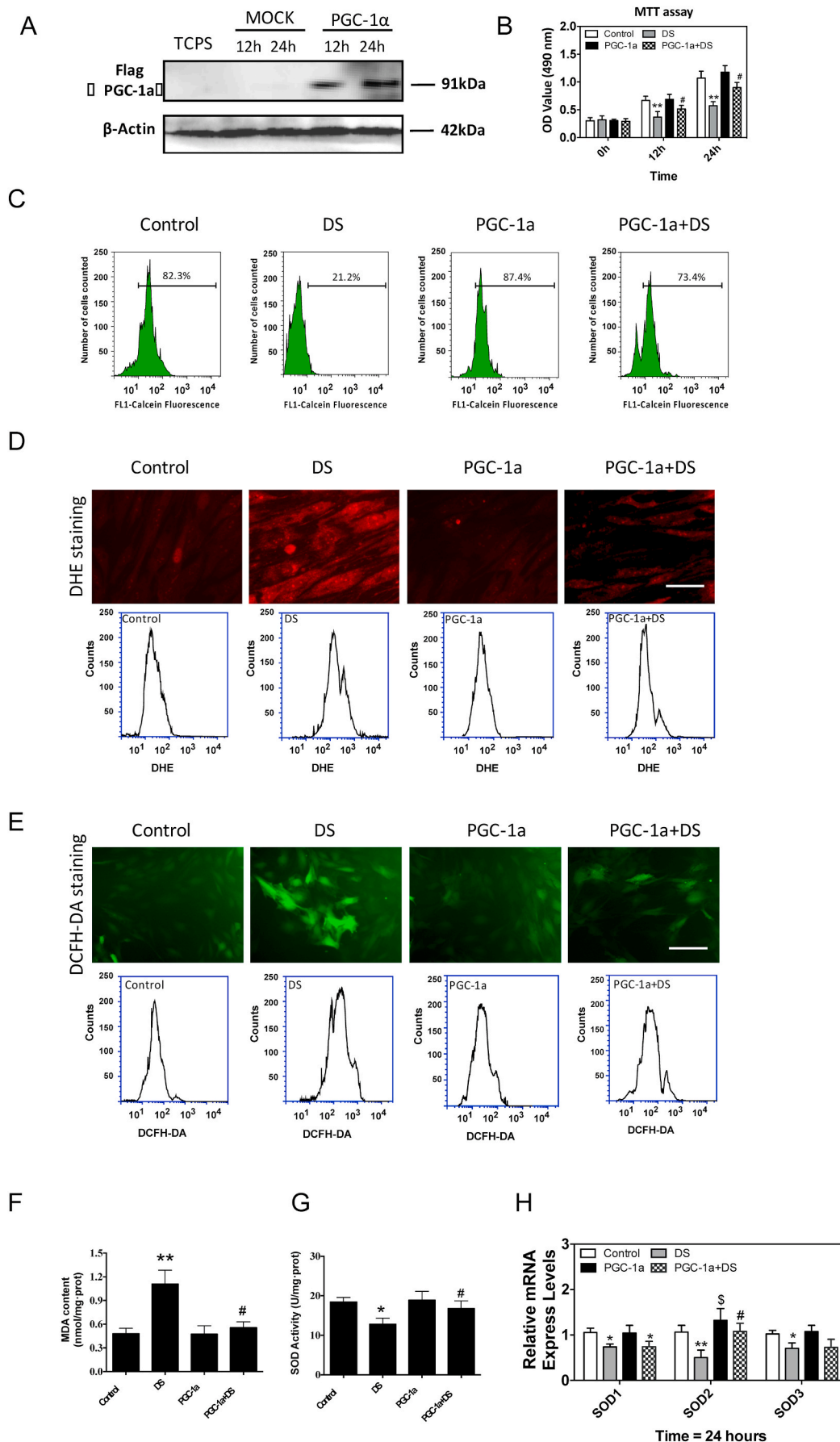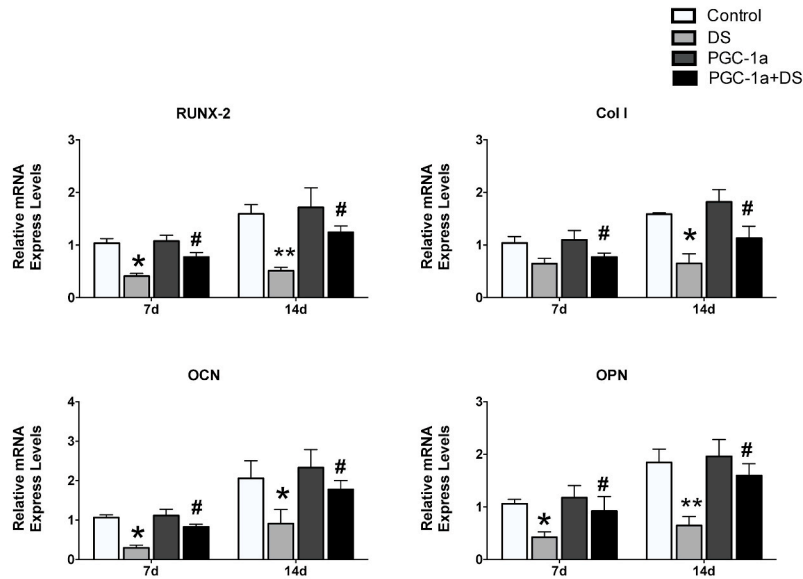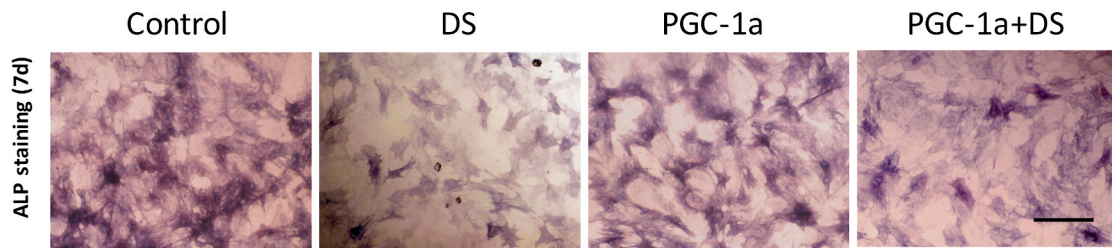
Fig. 2. Protective effect of PGC-1α in diabetic serum-mediated cellular injury. (A) Expression of PGC-1α in Mock and PGC-1α transfection MSCs and the protein expression (upper panel) of c-terminal Flag tagged PGC-1α was assessed with anti-Flag; (B) Cell viability of MSCs at 0, 12 and 24 h post diabetic serum treatment; (C) flow cytometer analysis of mitochondrial permeability transition pore (mPTP) opening after 24 h of incubation; **$P < 0.01$, vs. Control and PGC-1α Groups, #$P < 0.05$ vs.DS Group; (D) DHE staining and flow cytometer analysis of H_2O_2 levels; (E) DCFH-DA staining and flow cytometer analysis of intracellular total ROS levels after 24 h of incubation; (F) MDA, (G) total SOD activity analysis, and SOD1, 2, 3 genes mRNA expression levels measurement of MSCs in diabetic serum-mediated cellular injury after 24 h of incubation; *$P < 0.01$ and **$P < 0.01$, vs. Control and PGC-1α Groups, \$$P < 0.05$ vs. Control Group, #$P < 0.05$ vs.DS Group. Scale bar = 50 μm.
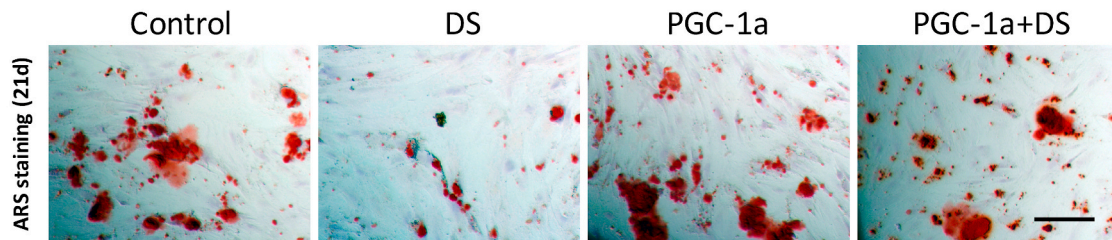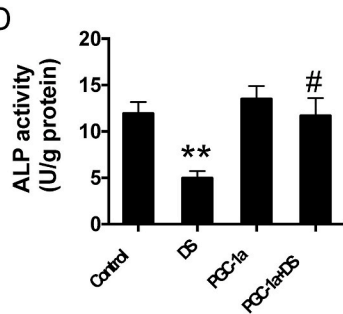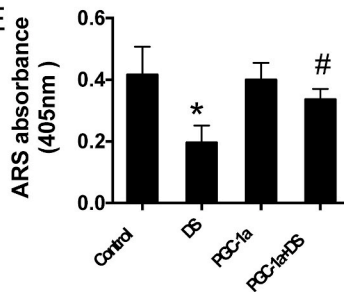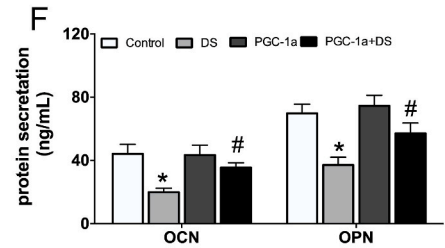
Fig. 3. Protective effect of PGC-1α over-expression on MSCs differentiation in diabetic serum-mediated cellular injury. **(A)** Relative osteogenic-specific genes RUNX2, Col 1, OCN and OPN mRNA expressions of MSCs. *P < 0.01 and **P < 0.01, vs. Control and PGC-1α Groups, #P < 0.05 vs.DS Group; (B) ALP staining and (D) ALP activity of MSCs after culture for 7 days; (C) ARS staining and (E) quantitative analysis for mineralization assay of MSCs at day 21 were measured; secreted OCN (F) and OPN were measured via ELISA. *P < 0.01 and **P < 0.01, vs. Control and PGC-1α Groups, #P < 0.05 vs.DS Group. Scale bar = 200 μm.

3.5. PGC-1α overexpression attenuated the diabetic serum-induced decreased angiogenesis regulation effects of MSC

To further analyze the protective effect of PGC-1 α over-expression in

diabetic serum-induced suppressed angiogenic regulation effects of MSCs, we firstly studied the mRNA expression of angiogenic factors via qRT-PCR. As shown in Fig. 4A, even though diabetic serum treatment significantly decreased the expression levels of VEGF-α, PDGF-bb and
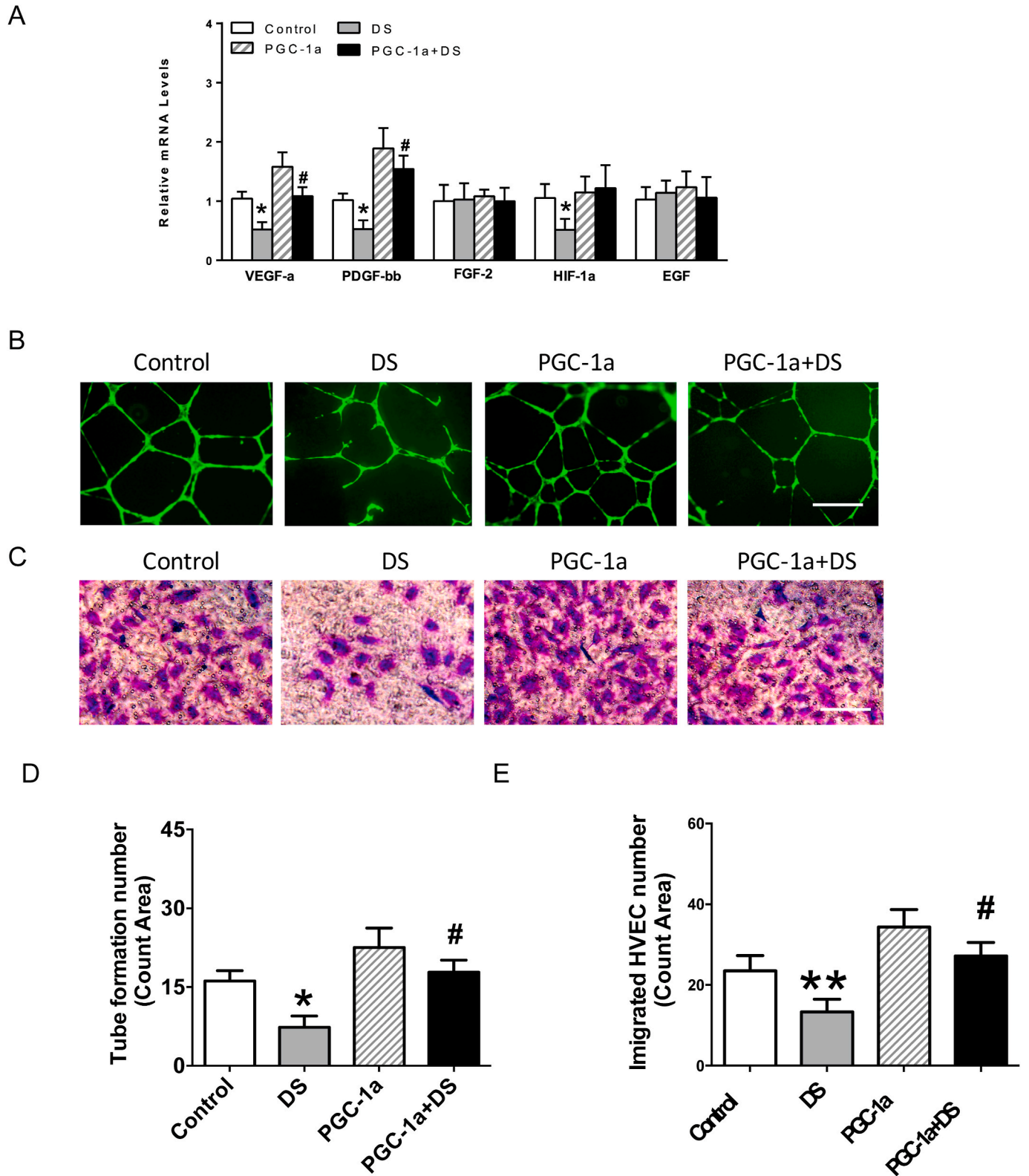

Fig. 4. The pro-angiogenic regulation effects of mesenchymal stem cell. (A) Relative Pro-angiogenic genes VEGF-enicenicfects of mesench α and EGF mRNA expressions of MSCs were measured via qRT-PCR; (B & D) HUVECs tube formation and (C & E) cellular immigration evaluation under MSCs conditioned media from different groups; *P < 0.01 and **P < 0.01, vs. Control and PGC-1α Groups, #P < 0.05 vs.DS Group. Scale bar = 100 μm.

HIF-1α (p < 0.05) in DS group as compared with Control group or PGC-1α group, PGC-1 α over-expression could only increase the expression levels of VEGF-α and PDGF-bb under diabetic serum treatment condition.

To further investigate the pro-angiogenic regulation effects of MSCs, the tube formation and immigration ability of HUVECs in MSC - conditioned medium were evaluated. As shown in Fig. 4 B - E, both tube formation number and immigration HUVECs number were significantly decreased in conditioned medium derived from DS group as compared with that from Control group (p < 0.05 or 0.01). Whereas, PGC-1 α over-expressed MSCs derived conditioned medium was proved to significantly alleviate down-regulated effects of tube formation number and immigration ability under diabetic serum environment in PGC-1 α + DS group. These findings suggested that diabetic serum decreased the angiogenic genes expression of MSCs as well as its effects on pro-angiogenic regulation, while it could be attenuated via PGC-1 α over-expression.

4. Discussion

Oxidative stress conditions induced by diabetes, inflammation, orthopedic surgery and bone fracture have been proved to be associated with impaired bone regenerative capacity [18–20]. In the current study, we found that PGC-1α overexpression demonstrated improved cyto-protective effects for defending MSCs from oxidative stress injury and contributed to enhanced osteogenic differentiation under diabetic condition. Moreover, PGC-1α overexpression in MSCs were also showed to attenuate its oxidative impaired pro-angiogenic capacity.

Mitochondria play a major role to counteract the harmful effects of oxidative damage, and the PGC-1α is a master regulator of mitochondrial biogenesis and oxidative metabolism to scavenge excessive ROS during the process [21]. Recently experiments had proved that dysregulated PGC-1a is associated with many pathogenesis, including Bone-Fat imbalance, insulin resistance, impaired mitochondrial fusion–fission homeostasis, chronic kidney disease and cardiomyopathy [13,22]. Although some previous studies proved that oxidative damage induced by simply administration of classic oxidative stressors like hydrogen peroxide (H_2O_2) could increase the expression of PGC-1α [23], some clinical studies also suggested that the diabetes is associated with decreased PGC-1α expression [24,25], which indicated regulation of PGC-1α expression under classic oxidative stressors and diabetes were in differently manner. Our results indicated that the PGC-1α expression decreased significantly under diabetic serum condition over time and the decreased PGC-1α expression could be remarkably restored via adding antioxidant NAC. These results indicated that PGC-1α could serve as a potential target to enhance anti-oxidative protective effect of MSCs following diabetic serum-induced cell injury.

Diabetes-induced oxidative stress can damage of cellular structures, lipids, proteins and nucleic acids, which contributed to the both mitochondrial damage and cellular dysfunction [26]. Consistently, our results proved that diabetic serum treated MSCs were observed with decreased cell viability, disrupted Mitochondrial Permeability Transition Pore (mPTP), increased intracellular ROS levels as well as decreased osteogenic differentiation ability. It has been well proved that PGC-1α overexpression has a protective effect against oxidative stress-induced cellular damage [27]. Our work demonstrated that PGC-1α over-expression could counteract diabetic serum -induced detrimental effects via anti-oxidative effects and SOD2 gene might play an important role in it. Interestingly, although PGC-1α overexpression was proved to increase the MSCs' mRNA expression level of SOD2 gene under normal condition, our data showed that the total SOD activity of PGC-1α overexpressed MSCs remains within normal range. These results suggested that the regulation effect of PGC-1α overexpression on total SOD activity under normal condition might involve more than expression levels of SOD2 enzymes [28]. As a result, PGC-1α overexpression was demonstrated to attenuate the impaired osteogenesis of MSCs elicited by excessive oxidative stress.

Bone regeneration involves a complex series of biological events and cell-to-cell communication among diverse cell-types, and it is well-documented that coupled osteogenesis and angiogenesis play a major role in bone growth, development and regeneration [29,30]. Recently studies also proved that diabetic conditions adverse affected angiogenic regulators expression as well as the processes of angiogenesis [31]. However, the protective effect of PGC-1α overexpression on MSC's pro-angiogenic regulation effects under diabetic conditions and its underlying mechanism remains unknown. In our study, our results showed that diabetic serum treatment significantly decreased mRNA expression of angiogenic factors VEGF-α and PDGF-bb, but it could be increased via PGC-1 α over-expression under diabetic conditions. What's more, the diabetic serum treatment could significantly decrease the endothelial tube formation and immigration regulated effects of MSCs. However, the diabetic conditions elicited down-regulated angiogenic effects of MSCs were alleviated via PGC-1 α over-expression.

5. Conclusion

This study proved that oxidative stress induced by diabetes caused dysfunction of MSCs, and contributed to impaired osteogenesis differentiation and pro-angiogenic capacity of MSCs. However, PGC-1α overexpression improved cyto-protective effects for defending MSCs from oxidative stress injury and contributed to attenuate its impaired osteogenic differentiation ability as well as pro-angiogenic capacity under diabetic conditions. Our findings indicated the great clinical potentials of PGC-1α regulation in MSCs for improved bone regeneration in patients with high levels of oxidative stress conditions.

Declaration of competing interest

The authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2021.101070.

Data availability

The statistical data of the article used to support the findings of this study are available from the corresponding author upon request.

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