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ORIGINAL ARTICLE

Stem cell secretome restore the adipo-osteo differentiation imbalance in diabetic dental pulp-derived mesenchymal stem cells

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Abstract

Background: Mesenchymal stem cells (MSCs) from type 2 diabetes mellitus (T2DM) individuals exhibit increased adipogenesis and decreased osteogenesis. We investigated the potential of adipose tissue-derived MSCs (ADMSCs) secretome obtained from healthy individuals in restoring the tumor necrosis factor-α (TNF-α) mediated imbalance in the adipo/osteogenic differentiation in the dental pulp-derived MSCs obtained from T2DM individuals (dDPMSCs).

Methods: dDPMSCs were differentiated into adipocytes and osteocytes using a standard cocktail in the presence of (a) induction cocktail, (b) induction cocktail + TNF-α, and (c) induction cocktail+ TNF-α + ADMSCs-secretome (50%) for 15 and 21 days resp. Differentiated adipocytes and osteocytes were stained by oil red O and alizarin red and analyzed by using ImageJ software. Molecular expression of the key genes involved was analyzed by using reverse-transcription polymerase chain reaction (RT-PCR).

Results: Treatment of TNF-α augmented the adipogenesis (9571 ± 765 vs. $19{,}815 \pm 1585$ pixel, $p < 0.01$) and decreased the osteogenesis ($15{,}603 \pm 1248$ vs. $11{,}894 \pm 951$ pixel, $p < 0.05$) of dDPMSCs as evidenced by the oil red O and alizarin red staining respectively. Interestingly, dDPMSCs differentiated along with TNF-α and 50% ADMSCs secretome exhibited enhanced osteogenesis ($11{,}894 \pm 951$ vs. $41{,}808 \pm 3344$ pixel, $p < 0.01$) and decreased adipogenesis ($19{,}815 \pm 1585$ vs. 4480 ± 358 pixel, $p < 0.01$). Additionally, dDPMSCs differentiated along with ADMSCs secretome exhibited decreased expression of PPARg ($p < 0.01$), C/EBPa ($p < 0.05$), and FAS ($p < 0.01$) whereas mRNA expression of Runx2 ($p < 0.05$), Osterix ($p < 0.01$), and OCN ($p < 0.05$) was upregulated as revealed by the RT-PCR analysis.

Conclusion: ADMSCs secretome from healthy individuals restore the TNF-α influenced differentiation fate of dDPMSCs and therefore can be explored for T2DM clinical management in the future.

KEYWORDS

differentiation, mesenchymal stem cells, microenvironment, secretome, type 2 diabetes mellitus

Key points

- Mesenchymal stem cells (MSCs) from type 2 diabetes mellitus (T2DM) individuals are known to exhibit increased adipogenesis and decreased osteogenesis.
- Adipose tissue-derived MSCs (ADMSCs) secretome from healthy individuals modulate the TNF-α influenced differentiation fate of MSCs obtained from diabetic individuals into adipocytes and osteocytes.
- ADMSCs secretome can be explored in restoring the disturbed microenvironment of MSCs and offering an effective alternative for the T2DM in future.

1 | INTRODUCTION

Bone is one of the key organs which provide support and protect the vital organs inside body. In the pathophysiological state of type 2 diabetes mellitus (T2DM), the microenvironment is known to influence bone homeostasis by modulating osteoclast/osteoblast ratio.[1] Clinical evidence suggest that T2DM influences skeletal metabolism and patients with uncontrolled hyperglycemia exhibit low bone mineral density and increased risk of fracture.[2–4] Enhanced osteoblast apoptosis, decline in the osteoblast differentiation, and enhanced bone resorption are the key hallmarks of the T2DM affected individual especially in the elderly population.[5]

Mesenchymal stem cells (MSCs) serve as precursor to various cell types. Interestingly, the fate of MSCs is decided by the chemical and physical microenvironment, and other factors such as age and metabolic status.[6] The fate of MSCs towards osteocytes and adipocytes is known to be implicated in several pathological conditions including obesity and T2DM.[6,7] Moreover, T2DM and metabolic syndrome have been predicted to be implicated in affecting MSCs properties and their subsequent differentiation fate. Serum of diabetic patients has been shown to impair the proliferation and differentiation of MSCs into osteocytes.[4] Additionally, serum obtained from diabetic individuals inhibit the osteogenic differentiation.[4] It is noteworthy that the MSCs population inside bone marrow tends to get differentiated into adipocytes rather than osteocytes in diabetic individuals.[8,9] The microenvironment in T2DM is comprised of pro-inflammatory cytokines, growth factors, triglycerides, hormones, and so forth.[10] Interestingly, high levels of TNF-α found in the serum of T2DM individuals are known to inhibit osteogenesis, and chondrogenesis, and also alter the miRNA composition in MSCs derived exosomes.[11]

Accumulating evidence suggests the therapeutic role of MSCs and their secretome in the clinical management of T2DM and bone disorders. In this study, we investigated whether secretome of MSCs from healthy individuals can restore the TNF-α mediated osteo/adipogenic differentiation imbalance in the MSCs from diabetic individuals.

2 | MATERIALS AND METHODS

2.1 | Mesenchymal stem cells isolation

Adipose-derived MSCs (ADMSCs) were procured from School of Regenerative Medicine, Bangluru (India). Dental pulp MSCs were isolated from the extracted tooth of diabetic patient having history of diabetes for more than 3 years (HbA1c > 9.0%) by explant culture as previously demonstrated by us.[12] DP-MSCs and dDPMSCs were expanded in the α-minimal essential medium (MEM) (Gibco), 10% fetal bovine serum (FBS, Gibco), and 1% antibiotic-antimycotic solution (Gibco).

2.2 | Stem cells characterization

Passage 4 ADMSCs and dDPMSCs were characterized for the positive cell surface marker expression of CD90, CD73, CD105 (1:1000, PE-Tagged), and negative cell surface marker expression of CD34, CD45, and HLADR (1:1000, FITC-Tagged) by using Attune NxT Flow Cytometer (Thermo Fisher Scientific) as shown by us.[13] Cells were further characterized for the tri-lineage potential by using differentiation cocktail for adipogenesis, osteogenesis, and chondrogenesis followed by staining with oil red O, alizarin red, and alcian blue staining as previously described.[12]

2.3 | Preparation of ADMSCs secretome (ADMSCs-S)

ADMSCs-S were obtained by the method described previously.[14] Passage 4-5 ADMSCs were seeded in T75 cell culture flask (1×10^6 cells per flask). Once MSCs attained 80%–85% confluence, they were washed with phosphate-buffered saline (PBS), and fresh α-MEM (without serum) was added. Culture flasks were kept at 37°C inside CO_2 incubator for 48 h. The secretome obtained was filtered by 0.22 μmol/L filters. ADMSCs-S was preserved in −80°C until further use.

2.4 | Analysis of growth factors and cytokines in the ADMSCs-S

ADMSCs-S obtained as described in the Section 2.3 was analyzed for presence of the growth factors and cytokine by using LEGEND plex™ Human Growth Factor Panel (13-plex) and MACS Plex cytokine 12 assay kit (Miltenyi Biotec) on Attune NxT Flow Cytometer as per the manufacturer's instruction.

2.5 | Adipogenic differentiation of dDPMSCs

For adipogenic differentiation, 2×10^4 cells were seeded in 24 well culture plates. Once they achieve 80%–90% confluency, they were given treatment of (a) adipogenic induction cocktail (dexamethasone [1 μmol/L], isobutyl methylxanthine [0.5 mmol/L], indomethacin [200 μmol/L], and insulin [10 μg]), (b) adipogenic induction cocktail + TNF-α, and (c) adipogenic induction cocktail + TNF-α + ADMSCs-CM (50%) for 15 days. At 15 days, the induction medium was discarded and cells were fixed with 4% paraformaldehyde for 30 min. After PBS wash, oil red O stain was added for 30 min. Oil red O stained area were quantitatively analyzed by using ImageJ software.

2.6 | Osteogenic differentiation of dDPMSCs

dDPMSCs were induced into osteocytes by using an induction cocktail (dexamethasone [0.1 μmol/L], β-glycerophosphate [10 mmol/L], and ascorbic acid [2 mmol/L]). Cells were given treatment of (i) induction cocktail (Control), (ii) induction cocktail + TNF-α, and (iii) induction cocktail + TNF-α + ADMSCs-CM (50%) for 21 days. The culture medium was changed twice a week. At 21 days, the induction medium was discarded followed by the fixation with 4% paraformaldehyde for 30 min. Further, cells were stained with alizarin red for 15 min, washed with distilled water, and visualized under microscope. Alizarin red stained areas were quantitatively analyzed by using ImageJ software.

2.7 | Alkaline phosphatase activity

Differentiation of dDPMSCs was performed as shown in Section 2.6 under the treatment of (i) induction cocktail (Control), (ii) induction cocktail + TNF-α, and (iii) induction cocktail + TNF-α + ADMSCs-CM (50%). At the end of 21 days, culture supernatant was collected and subjected to the alkaline phosphatase assay using QuantiChrom™ ALP assay kit described previously.[15] ALP activity of the control and treated samples were expressed as IU/L. ALP activity was calculated as follows.

$$\text{ALP Activity in IU/L} = \frac{(OD_{SAMPLE\,t} - OD_{SAMPLE\,0}) \cdot \text{Reaction Vol}}{(OD_{CALIBRATOR} - OD_{H_2O}) \cdot \text{Sample Vol} \cdot t} \times 35.3$$

2.8 | Gene expression studies

dDPMSCs were differentiated along with (i) induction cocktail (Control), (ii) induction cocktail + TNF-α, and (iii) induction cocktail + TNF-α + ADMSCs-CM (50%) into adipocytes and osteocytes for 15 and 21 days respectively as described in the Sections 2.5 and 2.6. TRIZOL method was adopted for total RNA extraction. Furthermore, total RNA was converted to cDNA by high capacity reverse transcription kit (Applied Biosciences). RT-PCR conditions and primer sequences of GAPDH, C/EBPα, PPARγ, FAS, Osteonectin, ALP, and RunX2 are given in Table 1. Data were normalized GAPDH expression levels. Relative quantification using the ΔΔCt method was used for data analysis.[16] RT-PCR analysis was executed on quantstudio 5 (Applied Biosystems).

2.9 | Statistical analysis

Statistical analysis was used by using SPSS (IBM) 20.0 software. One-way analysis of variance was performed to find differences in the treatment groups. *p*-Value less than 0.05 (*) and 0.01 (**) was considered significant. Experiments were performed in triplicates.

TABLE 1 Primer sequences used in the study.

Gene	Species	Forward	Reverse	Tm (°C)
GAPDH	Human	TCCCTGAGCTGAACGGGAAG	GGAGGAGTGGGTGTCGCTGT	60
C/EBPα	Human	GGGTCTGAGACTCCCTTTCCTT	CTCATTGGTCCCCCAGGAT	60
PPARg	Human	GAACGACCAAGTAACTCTCCTCAAAT	TCTTTATTCATCAAGGAGGCCAGCATT	62
FAS	Human	TATGCTTCTTCGTGCAGCAGTT	GCTGCCACACGCTCCTCTAG	60
Osteonectin	Human	GAGGAAACCGAAGAGGAGG	GGGGTGTTGTTCTCATCCAG	60
ALP	Human	GACGGACCCTCGCCAGTGCT	AATCGACGTGGGTGGGAGGGG	60
RunX2	Human	GGTTAATCTCCGCAGGTCAC	GTCACTGTGCTGAAGAGGCT	60

Abbreviation: Tm, temperature.

3 | RESULTS

3.1 | Characterization of MSCs

Post expansion of ADMSCs and dDPMSCs, cells exhibited fibroblast-like morphology. Furthermore, ADMSCs and dDPMSCs were positive for the expression of CD90, CD73, and CD105, whereas they lacked the expression of HLA-DR, CD45, and CD34 as shown in Figures 1 and 2, respectively. ADMSCs and dDPMSCs were able to differentiate into adipocytes, osteocytes and chondrocytes as evidenced by the oil red O, alizarin red and alcian blue staining.

3.2 | Growth factor and cytokine analysis of ADMSCs secretome

MSCs exhibit paracrine action through secretion of growth factors, cytokines, small bioactive proteins, etc. We found significant presence of the G-CSF, HGF, M-CSF, PDGF-AA, PDGF-BB, TGFa, VEGF, SCF, Angiopoetin-2, EGF, and EPO in the secretome of ADMSCs. Further, immunomodulatory cytokines such as CXCL8, IL-6, IL-4, CXCL-10, IL-1b, CCL2, and IL-17 were detected as shown in Figure 3.

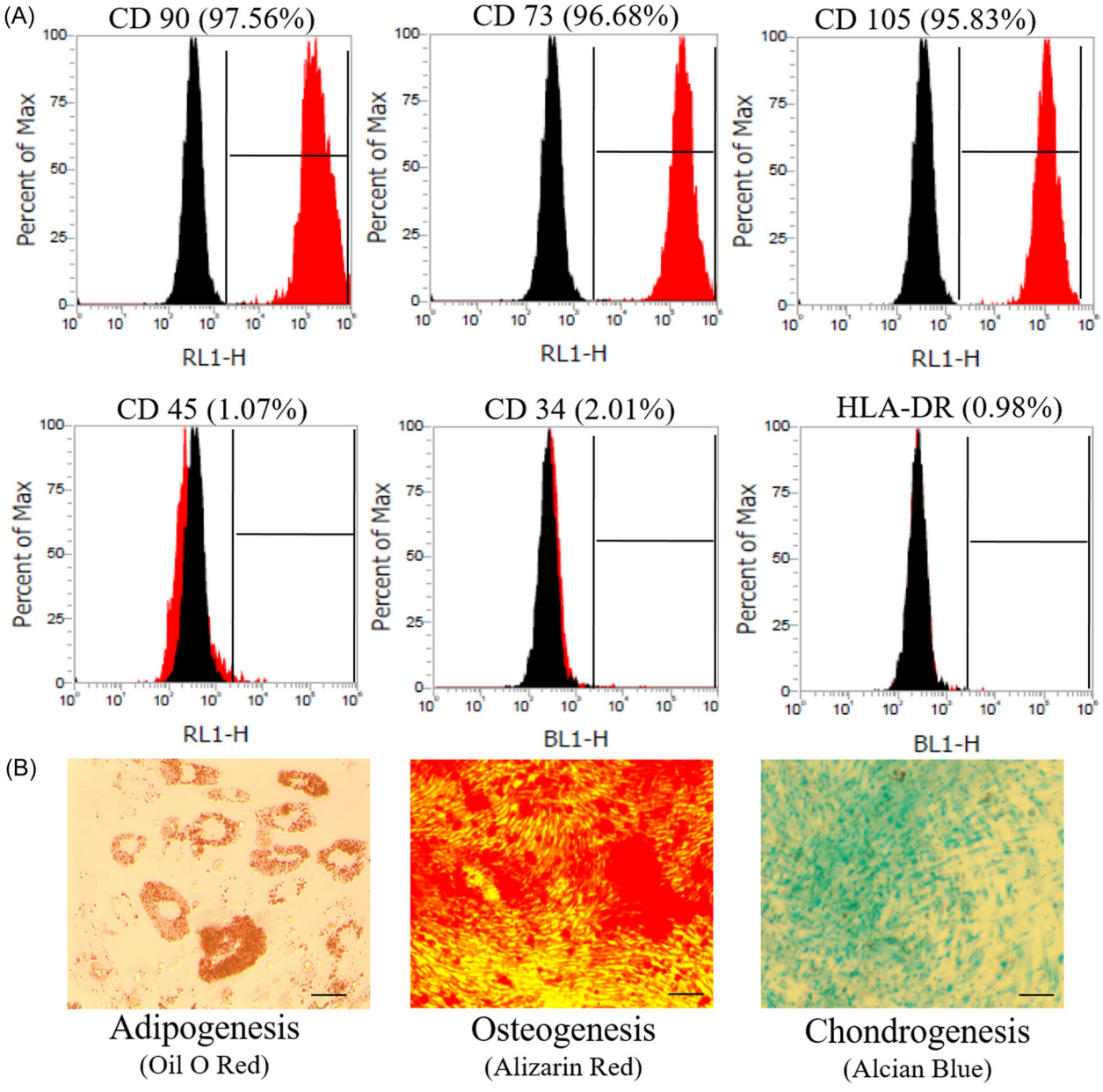

FIGURE 1 ADMSCs characterization: (A) ADMSCs showed positive (>95%) CD90, CD73, and CD105 expression whereas negative (<2%) CD45, CD34, and HLA-DR expression (B) Tri-lineage differentiation potential of ADMSCs, scale bars = 100 μm. ADMSCs, adipose tissue-derived mesenchymal stem cells.

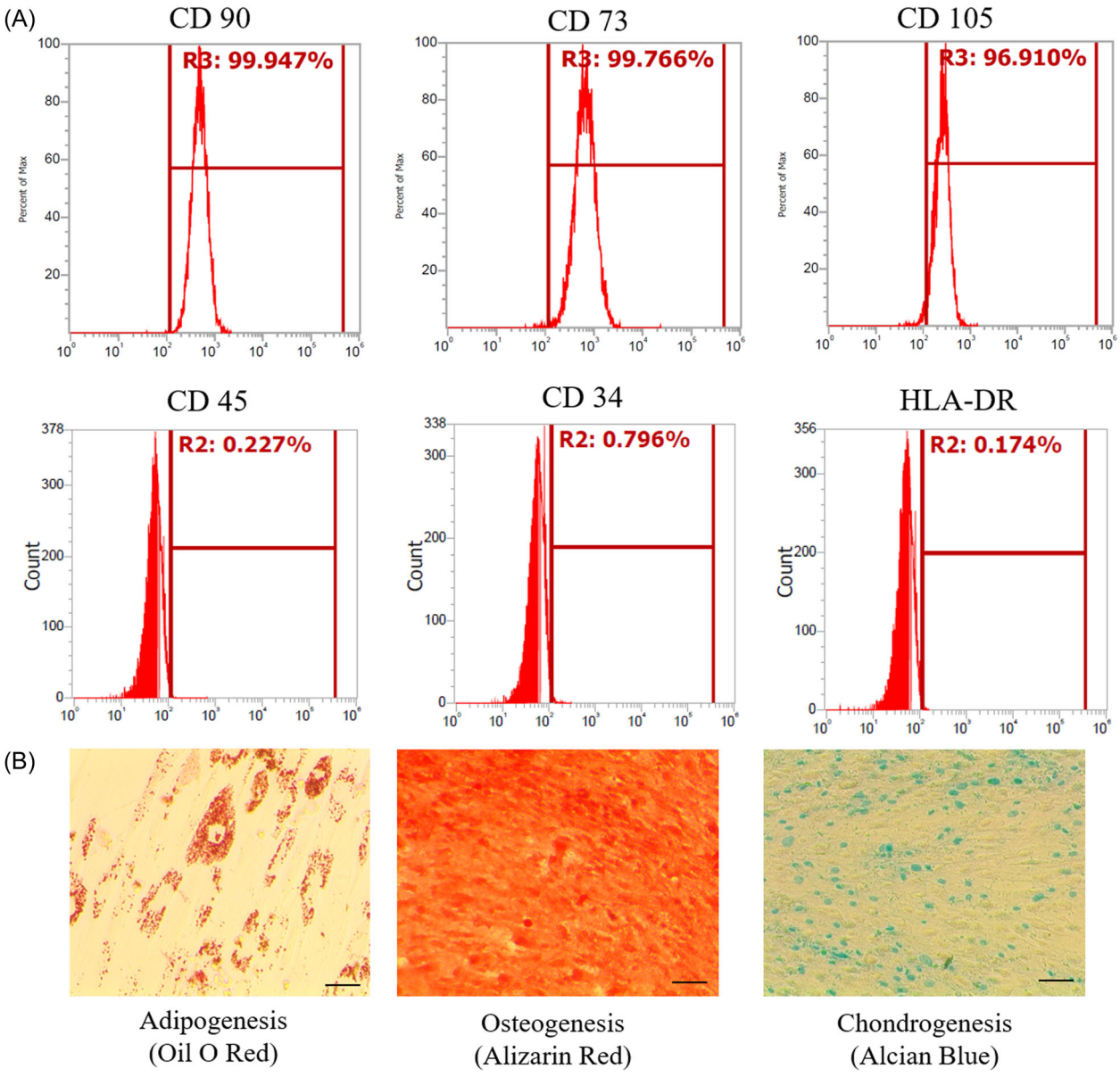

FIGURE 2 dDPMSCs characterization: (A) dDP-MSCs showed positive (>95%) CD90, CD73, and CD105 expression whereas negative (<2%) of and CD45, CD34, and HLA-DR expression. (B) Tri-lineage differentiation potential of dDPMSCs, scale bars = 100 μm. dDPMSCs, dental pulp-derived mesenchymal stem cells.

3.3 | ADMSCs secretome restore the TNF-α induced adipo-osteo imbalance in dDPMSCs

Our primary aim was to study the effect of ADMSCs secretome on the TNF-α influenced imbalance in osteogenic and adipogenic differentiation of dDPMSCs. The treatment of TNF-α resulted into decreased osteogenesis (15,603 ± 1248 vs. 11,894 ± 951 pixel, $p < 0.05$) and increased adipogenesis (9571 ± 765 vs. 19,815 ± 1585 pixel, $p < 0.01$) as evidenced by the alizarin red and oil red O staining (Figure 4A). Interestingly, dDPMSCs differentiated along with TNF-α and 50% ADMSCs secretome exhibited enhanced osteogenesis (11,894 ± 951 vs. 41,808 ± 3344 pixel, $p < 0.01$) and decreased adipogenesis (19,815 ± 1585 vs. 4480 ± 358 pixel, $p < 0.01$). Microscopic results were further validated with imageJ analysis of the alizarin red and oil red O staining and revealed the similar results (Figure 4B).

3.4 | ADMSCs secretome restore TNF-α mediated decrease in the ALP activity

ALP activity is the key indicator of the bone mineralization. Our results showed that the treatment of TNF-α resulted into significant decrease in the ALP activity (97.5 ± 6.3 vs. 57.5 ± 3.5 U/mL, $p < 0.01$), whereas ADMSCs secretome prevented the TNF-α mediated decrease in the ALP activity (57.5 ± 3.5 vs. 76.0 ± 4.2 U/mL, $p < 0.01$) as shown in Figure 5.

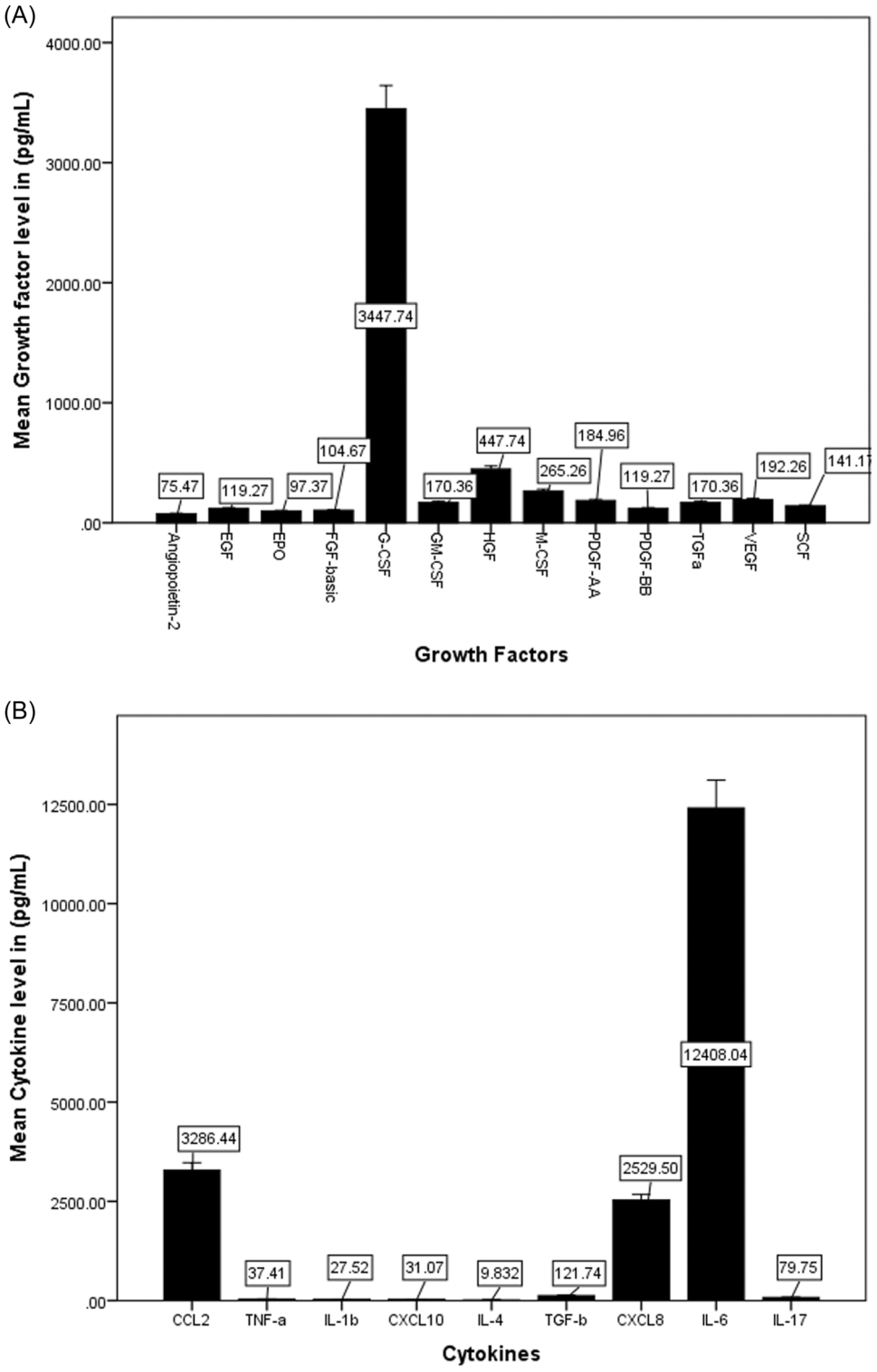

FIGURE 3 Levels of the growth factors (A) and cytokines (B) in the ADMSCs secretome analyzed by the FACS. Data shown are mean ± SD. ($n = 3$). ADMSCs, adipose tissue-derived mesenchymal stem cells; FACS, fluorescence activated cell sorting; SD, standard deviation.

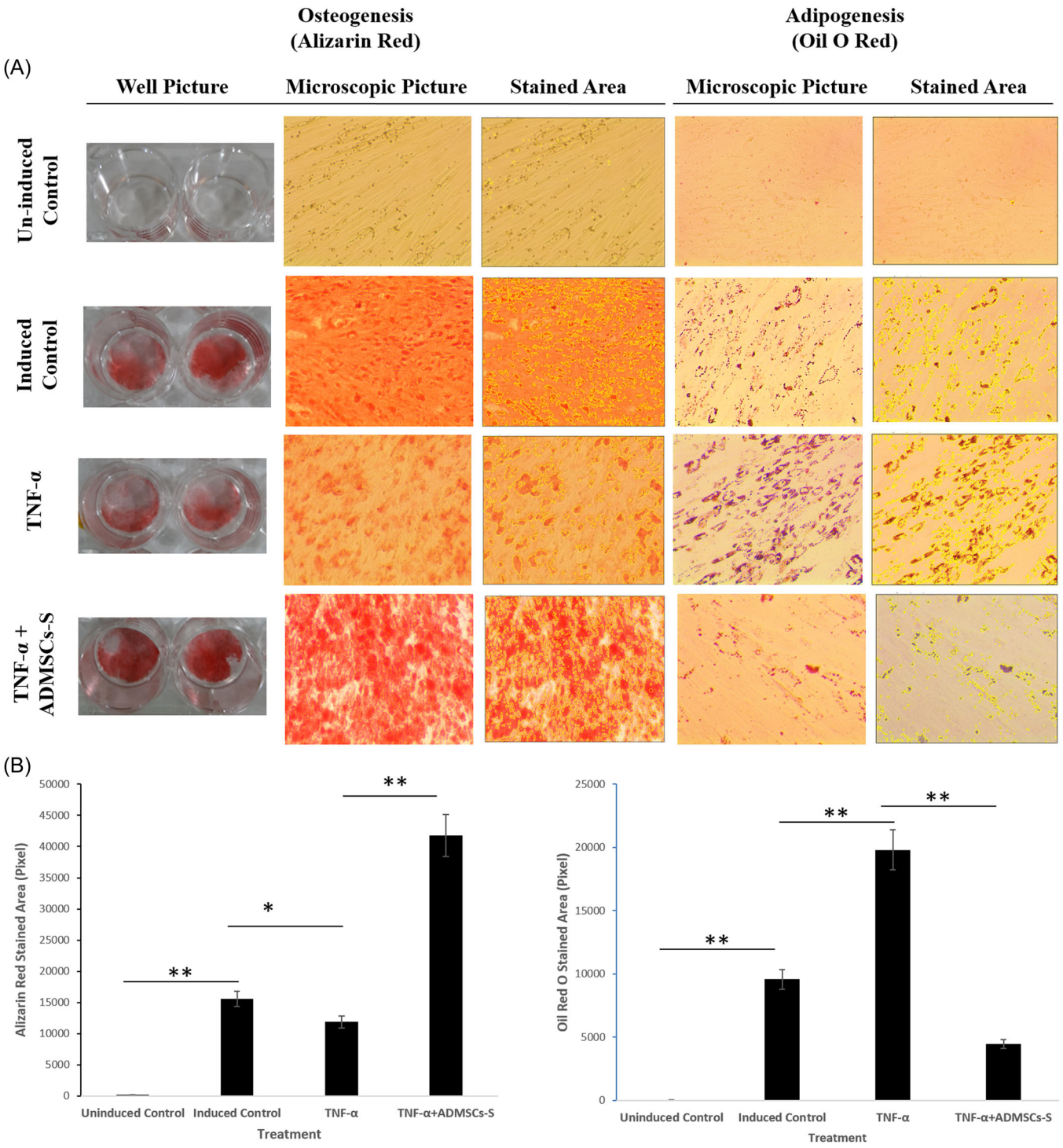

FIGURE 4 (A) Osteogenic and adipogenic differentiation of ADMSCs in presence of (i) induction cocktail (Control), (ii) induction cocktail + TNF-α, and (iii) induction cocktail + TNF-α + ADMSCs-CM (50%). Differentiated osteocytes and adipocytes were stained with alizarin red and oil red O stain, respectively. ($n = 3$). Stained area is selected with ImageJ analysis software (yellow boundary) for the quantitative analysis. Images are of 4X resolution, (B) ImageJ analysis of the alizarin red and oil red O stained area, ($n = 3$, $p < 0.05$, $p < 0.01$). ADMSCs, adipose tissue-derived mesenchymal stem cells; TNF-α, tumor necrosis factor-α.

3.5 | ADMSCs secretome restore TNF-α mediated gene expression in the differentiated dDPMSCs

To further validate our results, we analyzed the mRNA transcript expression implicated in adipogenesis (PPARg, C/EBPa, and FAS) and osteogenesis (Runx2, Osterix, and OCN) by RT-PCR. We observed a significant up regulation of the mRNA expression of PPARg ($p < 0.01$), C/EBPa ($p < 0.01$), and FAS ($p < 0.01$) upon treatment with TNF-α whereas mRNA expression of Runx2 ($p < 0.01$), Osterix ($p < 0.01$), and OCN ($p < 0.05$) was significantly down

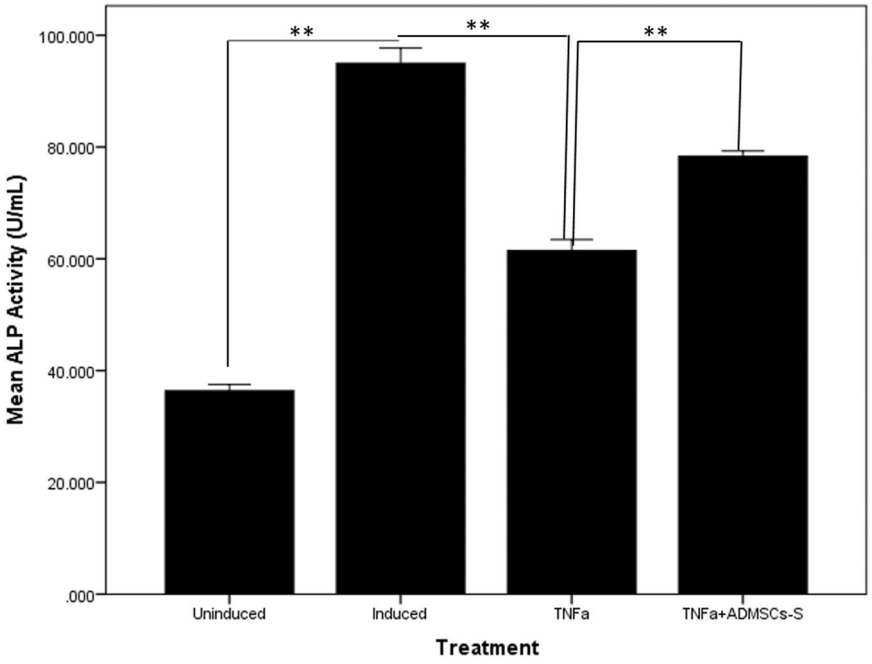

FIGURE 5 ALP activity of the differentiated dDPMSCs in presence of (i) induction cocktail (Control), (ii) induction cocktail + TNF-α, and (iii) induction cocktail + TNF-α + ADMSCs-CM (50%). Data shown are mean ± SD. ($n = 3$, $p < 0.05$, $p < 0.01$). ADMSCs, adipose tissue-derived mesenchymal stem cells; ALP, alkaline phosphatase; SD, standard deviation; TNF-α, tumor necrosis factor-α.

regulated. Interestingly, dDPMSCs differentiated along with ADMSCs secretome exhibited decreased expression of PPARg ($p < 0.01$), C/EBPa ($p < 0.05$), and FAS ($p < 0.01$) whereas mRNA expression of Runx2 ($p < 0.05$), Osterix ($p < 0.01$), and OCN ($p < 0.05$) was upregulated (Figure 6).

4 | DISCUSSION

Our study unequivocally revealed that ADMSCs secretome derived from healthy individuals restore the TNF-α mediated imbalance in adipogenesis and osteogenesis of MSCs obtained from diabetic donor as evidenced by the oil red O and alizarin red staining resp. Furthermore, ADMSCs secretome potentially restored the TNF-α mediated decrease in the ALP activity which is one of the reliable markers of osteogenic differentiation. Our results were further validated with mRNA transcript expression which revealed that ADMSCs secretome significantly decreased the mRNA transcript expression of *PPARg*, C/EBPa and FAS whereas increase the expression of Runx2, Osterix and OCN in the differentiated dDPMSCs.

The secretome of MSCs is a unique composition of soluble growth factors and cytokines, micro vesicles, exosomes, small proteins and miRNAs. Additionally, MSCs secretome is known possess potent immunomodulatory potential which have been tested in varieties of inflammatory clinical conditions.[17,18] The secretome of ADMSCs possess high levels of G-CSF, HGF, M-CSF, PDGF-AA, PDGF-BB, TGFa, VEGF, SCF, angiopoetin-2, EGF, and EPO cytokines such as CXCL8, IL-6, IL-4, CXCL-10, IL-1b, CCL2, and IL-17. Interestingly, previous studies have shown that growth factors such as FGFs, PDGF, EGF, VEGF, and IGF modulate the adipogenic and osteogenic differentiation of MSCs.[19,20] The FGF family of proteins are known to modulate the osteogenic and adipogenic differentiation of MSCs.[19,21] Interestingly, FGF2 is known to induce expression RunX2, a key transcriptional regulator of osteogenesis in MSCs.[22] FGF2 is also known to enhance ALP activity and matrix mineralization in rat bone marrow precursor cells. Further, bone is a highly vascularized organ and angiogenesis is pivotal in the osteogenesis process. VEGF and PDGF is one of the principle growth factor secreted by MSCs known to enhance osteogenesis by modulation of inflammation, endochondral and intramembranous ossification.[23,24] VEGF is also implicated in determining the stem cells fate, especially into adipocytes and osteocytes through gene modulation of RunX2 and PPARγ transcript expression.[25] Furthermore, EGF is known to enhance osteogenic differentiation of DPMSCs through up regulated expression of ALP and osteocalcin.[26]

In the context of adipogenesis, our studies have shown that ADMSCs secretome inhibit the adipogenesis and ameliorate the insulin resistance in 3T3-L1 cells.[14,27] Additionally, ADMSCs secretome reduce the intramuscular accumulation of triglycerides accumulation in C2C12 cells.[14] In a high fat diet induced obese mice, ADMSCs reduce the body weight and improve the glucose homeostasis.[28] Moreover, ADMSCs improve the glucose homeostasis by enhancing glycogen synthesis and inhibition of hepatic glucose production in T2DM rats.[29] Interestingly, secretome obtained from human tonsil-derived

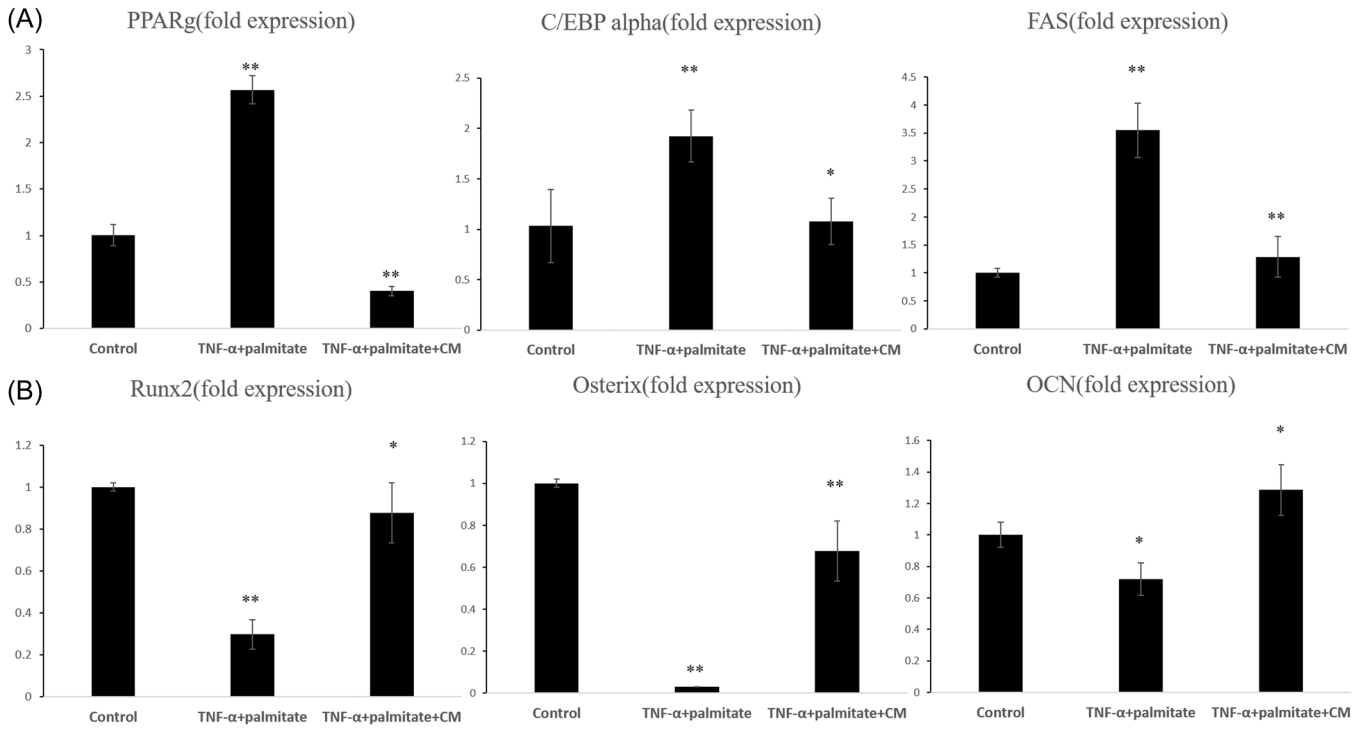

FIGURE 6 mRNA expression of gene implicated in (A) adipogenesis (PPARg, C/EBPa, and FAS) and (B) osteogenesis (Runx2, Osterix, and OCN) in the differentiated dDPMSCs along with (i) induction cocktail (Control), (ii) induction cocktail + TNF-α, and (iii) induction cocktail + TNF-α + ADMSCs-CM (50%) assessed by RT-PCR. ($n = 3$, $p < 0.05$, $p < 0.01$). ADMSCs, adipose tissue-derived mesenchymal stem cells; RT-PCR, reverse-transcription polymerase chain reaction; TNF-α, tumor necrosis factor-α.

MSCs (TMSCs) inhibit adipogenic differentiation via inhibition of glucocorticoid signaling.[30] Also, T-MSCs secretome reduced the visceral and bone marrow adiposity in the dexamethasone induced obesity in BALC/c mice.[30] It is noteworthy that the glucocorticoids which are widely prescribed drugs in inflammatory conditions can result into osteoporosis even in the young individuals.[31] From our study, it is evident that ADMSCs secretome possess immunomodulatory cytokines which have been shown to inhibit glucocorticoid induced adipogenesis via phosphorylation of p38 and glucocorticoid receptors.[30]

Our findings from this study possess immense clinical value as MSCs and their secretions are undergoing active clinical investigations in the T2DM and osteoporosis. The fate of MSCs under the influence of a diabetic microenvironment is compromised and inclined towards the adipocytes rather than osteocytes.[6] Therefore, application of secretome derived from ADMSCs to restore the fate of MSCs in T2DM microenvironment can be an effective strategy in the T2DM therapy.

In conclusion, our study unequivocally demonstrates that ADMSCs secretome obtained from healthy individuals restore the TNF-α mediated adipo-osteo differentiation imbalance in the dDPMSCs. The potential of ADMSCs secretome can be harnessed in the effective clinical management of T2DM via modulating the T2DM microenvironment.

AUTHOR CONTRIBUTIONS

Study conception and design: Avinash Sanap and Ramesh Bhonde. *Data collection*: Avinash Sanap, Kalpana Joshi, and Ramesh Bhonde. *Analysis and interpretation of results*: Avinash Sanap, Ramesh Bhonde, and Kalpana Joshi. *Draft manuscript preparation*: Avinash Sanap and Ramesh Bhonde. *Infrastructure access and analysis*: Kalpana Joshi and Supriya Kheur.

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CONFLICT OF INTEREST STATEMENT

All authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Upon reasonable request.

ETHICS STATEMENT

All study protocols involving the stem cell culture were approved by the Institutional Committee for Stem Cells Research of Sinhgad College of Engineering, Pune

(India). Informed consents were obtained from the individuals before the sample collection.

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REFERENCES

1. Xu Y, Wu Q. Trends in osteoporosis and mean bone density among type 2 diabetes patients in the US from 2005 to 2014. *Sci Rep*. 2021;11(1):3693. doi:10.1038/s41598-021-83263-4
2. Auwerx J, Dequeker J, Bouillon R, Geusens P, Nijs J. Mineral metabolism and bone mass at peripheral and axial skeleton in diabetes mellitus. *Diabetes*. 1988;37(1):8-12. doi:10.2337/DIAB.37.1.8
3. Abdulameer SA, Sulaiman SA, Hassali MAA, Subramaniam K, Sahib M. Osteoporosis and type 2 diabetes mellitus: what do we know, and what we can do? *Patient Prefer Adherence*. 2012;6:435-448. doi:10.2147/PPA.S32745
4. Deng X, Xu M, Shen M, Cheng J. Effects of type 2 diabetic serum on proliferation and osteogenic differentiation of mesenchymal stem cells. *J Diabetes Res*. 2018;2018:1-9. doi:10.1155/2018/5765478
5. Justesen J, Stenderup K, Ebbesen EN, Mosekilde L, Steiniche T, Kassem M. Adipocyte tissue volume in bone marrow is increased with aging and in patients with osteoporosis. *Biogerontology*. 2001;2(3):165-171. doi:10.1023/A:1011513223894
6. Chen Q, Shou P, Zheng C, et al. Fate decision of mesenchymal stem cells: adipocytes or osteoblasts. *Cell Death Differ*. 2016;23(7):1128-1139. doi:10.1038/cdd.2015.168
7. Pino AM, Rosen CJ, Rodríguez JP. In osteoporosis, differentiation of mesenchymal stem cells (MSCs) improves bone marrow adipogenesis. *Biol Res*. 2012;45:279-287.
8. Moseley KF, Doyle ME, Jan De Beur SM. Diabetic serum from older women increases adipogenic differentiation in mesenchymal stem cells. *Endocr Res*. 2018;43(3):155-165. doi:10.1080/07435800.2018.1441868
9. Kornicka K, Houston J, Marycz K. Dysfunction of mesenchymal stem cells isolated from metabolic syndrome and type 2 diabetic patients as result of oxidative stress and autophagy May limit their potential therapeutic use. *Stem Cell Rev Rep*. 2018;14(3):337-345. doi:10.1007/s12015-018-9809-x
10. Sanap A, Bhonde R, Joshi K. Mesenchymal stem cell conditioned medium ameliorates diabetic serum-induced insulin resistance in 3T3-L1 cells. *Chronic Dis Transl Med*. 2021;7(1):47-56. doi:10.1016/j.cdtm.2020.09.001
11. Li W, Liu Q, Shi J, Xu X, Xu J. The role of TNF-α in the fate regulation and functional reprogramming of mesenchymal stem cells in an inflammatory microenvironment. *Front Immunol*. 2023;14(February):1-14. doi:10.3389/fimmu.2023.1074863
12. Patil VR, Kharat AH, Kulkarni DG, Kheur SM, Bhonde RR. Long term explant culture for harvesting homogeneous population of human dental pulp stem cells. *Cell Biol Int*. 2018;42(12):1602-1610. doi:10.1002/cbin.11065
13. Sanap A, Chandravanshi B, Shah T, et al. Herbal pre-conditioning induces proliferation and delays senescence in Wharton's jelly mesenchymal stem cells. *Biomed Pharmacother*. 2017;93:772-778. doi:10.1016/j.biopha.2017.06.107
14. Shree N, Bhonde RR. Conditioned media from adipose tissue derived mesenchymal stem cells reverse insulin resistance in cellular models. *J Cell Biochem*. 2017;118(8):2037-2043. doi:10.1002/jcb.25777
15. Villa-Bellosta R, González-Parra E, Egido J. Alkalosis and dialytic clearance of phosphate increases phosphatase activity: a hidden consequence of hemodialysis. *PLoS One*. 2016;11(7):e0159858. doi:10.1371/journal.pone.0159858
16. Shah T, Mishra S, More A, Otiv S, Apte K, Joshi K. Combination of vitamin B12 active forms improved fetal growth in Wistar rats through up-regulation of placental miR-16 and miR-21 levels. *Life Sci*. 2017;191(October):97-103. doi:10.1016/j.lfs.2017.10.017
17. Wang Y, Fang J, Liu B, Shao C, Shi Y. Reciprocal regulation of mesenchymal stem cells and immune responses. *Cell Stem Cell*. 2022;29(11):1515-1530. doi:10.1016/j.stem.2022.10.001
18. Wang LT, Ting CH, Yen ML, et al. Human mesenchymal stem cells (MSCs) for treatment towards immune- and inflammation-mediated diseases: review of current clinical trials. *J Biomed Sci*. 2016;23(1):76. doi:10.1186/s12929-016-0289-5
19. Eswarakumar VP, Lax I, Schlessinger J. Cellular signaling by fibroblast growth factor receptors. *Cytokine Growth Factor Rev*. 2005;16(2):139-149. doi:10.1016/j.cytogfr.2005.01.001
20. Kratchmarova I, Blagoev B, Haack-Sorensen M, Kassem M, Mann M. Mechanism of divergent growth factor effects in mesenchymal stem cell differentiation. *Science*. 2005;308(5727):1472-1477. doi:10.1126/science.1107627
21. Jackson RA, Nurcombe V, Cool SM. Coordinated fibroblast growth factor and heparan sulfate regulation of osteogenesis. *Gene*. 2006;379(1-2):79-91. doi:10.1016/j.gene.2006.04.028
22. Woei Ng K, Speicher T, Dombrowski C, et al. Osteogenic differentiation of murine embryonic stem cells is mediated by fibroblast growth factor receptors. *Stem Cells Dev*. 2007;16(2):305-318. doi:10.1089/scd.2006.0044
23. Hu K, Olsen BR. The roles of vascular endothelial growth factor in bone repair and regeneration. *Bone*. 2016;91(2016):30-38. doi:10.1016/j.bone.2016.06.013
24. Grosso A, Burger MG, Lunger A, Schaefer DJ, Banfi A, Di Maggio N. It takes two to tango: coupling of angiogenesis and osteogenesis for bone regeneration. *Front Bioeng Biotechnol*. 2017;5:1-7. doi:10.3389/fbioe.2017.00068
25. Liu Y, Berendsen AD, Jia S, et al. Intracellular VEGF regulates the balance between osteoblast and adipocyte differentiation. *J Clin Invest*. 2012;122(9):3101-3113. doi:10.1172/JCI61209
26. Del Angel-Mosqueda C, Gutiérrez-Puente Y, López-Lozano AP, et al. Epidermal growth factor enhances osteogenic differentiation of dental pulp stem cells in vitro. *Head Face Med*. 2015;11(1):29. doi:10.1186/s13005-015-0086-5
27. Sanap A, Bhonde R, Joshi K. Conditioned medium of adipose derived mesenchymal stem cells reverse insulin resistance through downregulation of stress induced serine kinases. *Eur J Pharmacol*. 2020;881:173215. doi:10.1016/j.ejphar.2020.173215
28. Cao M, Pan Q, Dong H, et al. Adipose-derived mesenchymal stem cells improve glucose homeostasis in high-fat diet-induced obese mice. *Stem Cell Res Ther*. 2015;6(1):208. doi:10.1186/s13287-015-0201-3
29. Xie M, Hao HJ, Cheng Y, et al. Adipose-derived mesenchymal stem cells ameliorate hyperglycemia through regulating hepatic glucose metabolism in type 2 diabetic rats. *Biochem Biophys Res Commun*. 2017;483(1):435-441. doi:10.1016/j.bbrc.2016.12.125
30. Kim YH, Lee HJ, Cho KA, Woo SY, Ryu KH. Conditioned medium from human tonsilderived mesenchymal stem cells inhibits glucocorticoid-induced adipocyte differentiation. *PLoS One*. 2022;17(6):e0266857. doi:10.1371/journal.pone.0266857
31. Schacke H. Mechanisms involved in the side effects of glucocorticoids. *Pharmacol Ther*. 2002;96(1):23-43. doi:10.1016/s0163-7258(02)00297-8

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