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In Vitro Evaluation of Human Demineralised Teeth Matrix on Osteogenic Differentiation of Gingival Mesenchymal Stem Cells

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ABSTRACT

The use of tooth-derived material as a scaffold has gained attention recently due to its ease of availability and bioactive properties. Hence, the objective of this study was to determine in vitro interaction of human gingival mesenchymal stem cells (hGMSCs) with human demineralised teeth matrix (hDTM) on osteogenic potential with or without osteogenic inducers. The hGMSCs were established and characterised on their morphology, proliferation, population doubling time (PDT), viability, colony-forming ability, expression of cell surface markers and adipogenic differentiation. Further, the effect of hDTM on the biocompatibility and osteogenic differentiation ability of hGMSCs was evaluated. The hGMSCs displayed a fibroblast-like appearance and exhibited a greater proliferative activity. The cells showed > 91% viability, and PDT varied between 39.34 hours and 62.59 hours. Further, hGMSCs indicated their propensity to form clusters/colonies, and expressed the markers, such as CD29, CD44, CD73 and CD90, but were negative for CD34 and CD45. When treated with adipogenic induction medium, hGMSCs were able to exhibit the formation of neutral lipid vacuoles. The hGMSCs cultured with hDTM did not show any cytotoxic changes including morphology and viability. Mineralisation of calcium nodules was observed in hGMSCs when cultured in osteogenic induction (OI) medium as an indication of osteogenesis. hGMSCs when cultured with hDTM confirmed the presence of a mineralised matrix. Further, when the cells were cultured with hDTM along with OI, they showed slightly enhanced differentiation into osteocytes. In conclusion, hGMSCs were shown to be biocompatible with hDTM, and demonstrated their enhanced osteogenic potential in the presence of hDTM and osteogenic supplements.

Keywords: *Demineralised teeth matrix; gingival mesenchymal stem cells; human; in vitro; osteogenesis*

INTRODUCTION

Various pathologic lesions of jaws need partial and total resection which produce defects of varying sizes necessitating reconstruction for restoration of function and aesthetics (Mittal *et al.*, 2018). Autogenous bone grafting is the gold

standard for reconstruction of bone defects because of its excellent regenerative ability (Minamizato *et al.*, 2018), but has major drawbacks, such as high resorption rates, limited available sources and donor site morbidity (Akhlaghi *et al.*, 2019). Alternative graft materials, including allografts, xenografts and alloplastic bone grafts are also available, but they have high cost, limited osteoinduction capacity and carry the risk of disease transmission (Minamizato *et al.*, 2018). Due to these limitations, new options in regenerative medicine and tissue engineering are being explored for functional and anatomical restoration (Shakoori *et al.*, 2017). In bone tissue engineering, mesenchymal stem cell (MSC)-based approach has shown to be a viable alternative for clinical use (Al-Qadhi *et al.*, 2020; Kim *et al.*, 2021).

The easy accessibility, high proliferative ability and multi-lineage potential of human gingiva-derived MSCs (hGMSCs) make these cells as a promising source for bone tissue regeneration (Zhang *et al.*, 2009; Tomar *et al.*, 2010; Wang *et al.*, 2011; Kim *et al.*, 2021; Kumar *et al.*, 2021a). In addition, comparative characterisation of GMSCs with other sources, such as bone marrow-derived MSCs (BMSCs), periodontal ligament-derived stem cells (PDLSCs) and umbilical cord-derived-MSCs (UC-MSCs) has been shown to be similar or superior in terms of various cellular, biological and immunological properties (Zhang *et al.*, 2009; Tomar *et al.*, 2010; Al-Qadhi *et al.*, 2020; Kim *et al.*, 2021; Subba *et al.*, 2022). Further, a few pre-clinical studies have demonstrated the *in vivo* bone regeneration capacity of GMSCs (Wang *et al.*, 2011; Al-Qadhi *et al.*, 2020; Kim *et al.*, 2021).

In tissue engineering, scaffolds play a key role in the *in vivo* behaviour of GMSCs and can influence the final outcome of any regenerative therapy. Various cell delivery approaches are currently being used for GMSCs transplantation, including GMCSs sheet, biografts, such as hydroxyapatite/tricalcium phosphate (HA/TCP) (Zhang *et al.*, 2009; Tomar *et al.*, 2010; Kim *et al.*, 2021), scaffold-augmented GMSCs transplantation using collagen (Wang *et al.*, 2011), or fibrin (Tang *et al.*, 2011), gel matrix (Brizuela *et al.*, 2016) or electrospun polycaprolactone scaffolds (Jauregui *et al.*, 2018), and poly(lactide) (PLA) scaffolds (Diomede *et al.*, 2018). All these methods provide platforms for in vivo cell transplantation, but with limited osteoinduction and bioactivity.

The tooth-derived material as a scaffold has attracted attention in recent times, due to ease of availability of teeth which are extracted on frequent basis and discarded as biomedical waste (Kim *et al.*, 2010; Bono *et al.*, 2017; Kang *et al.*, 2017; Um *et al.*, 2017; Kumar *et al.*, 2021b). The level of growth factors are high in enamel and dentine matrix, and largely resemble the composition of bone matrix. Further, it has been opined that bone morphogenetic proteins (BMPs) in dentin and bone are the major factors with osteoinductive potential and mainly involve in bone formation (Kim *et al.*, 2010; Bono *et al.*, 2017; Kabir *et al.*, 2021). With these osteogenic properties, in vitro and in vivo studies have shown enhancement in adhesion, migration capacity and osteogenic differentiation of dental pulp stem cells (DPSCs) in the presence of demineralised dentin matrices (DDM) (Liu *et al.*, 2016). Recently, human demineralised teeth matrix (DTM)-derived conditioned media maintained its biocompatibility with DPSCs and enhanced the proliferation and osteogenic differentiation (Kumar *et al.*, 2021b). Except these, no studies have so far evaluated the cytotoxicity and osteogenic potential of hGMSCs in the presence of human demineralised teeth matrix (hDTM). Previous studies have shown the promising potential of GMSCs as a readily accessible and expandable source that can be easily obtained as a clinical waste by minimally invasive surgical procedures (Zhang *et al.*, 2009; Wang *et al.*, 2011; Kim *et al.*, 2021; Subba *et al.*, 2022). Therefore, the present study was aimed at determining the in vitro interaction and osteogenic differentiation

potential of GMSCs with hDTM prepared from non-carious teeth in the presence and absence of osteogenic inducers.

MATERIALS AND METHODS

Ethical Approval

Informed consent was obtained from patients, and the study followed the Declaration of Helsinki on medical protocol and ethics. The ethical approval for this in vitro study was obtained from the ethical committee of AB Shetty Memorial Institute of Dental Sciences (Ref.: ABSM/EC36/2017).

Isolation and Culture of hGMSCs

Gingival tissue samples were collected from patients with clinically healthy gingiva and no history of periodontitis. The collected tissues were immediately placed in a sterile vial containing Dulbecco's phosphate buffered saline (DPBS, Gibco, Thermo Fisher Scientific, Grand Island, NY, USA) with penicillin and streptomycin (Gibco). Under aseptic conditions, the collected gingival tissue was washed with DPBS thrice and with sterile scalpel, the tissue was sliced to 1 mm^3. The minced tissue was transferred into sterile tube for enzymatic digestion containing 0.1% collagenase enzyme (Gibco-Invitrogen) and was kept for 2 hours in humidified incubator of 5% CO_2 at 37°C. After the digestion process, complete media (DMEM-High Glucose, Gibco-Invitrogen) was added to neutralise the traces of enzyme. Then digested cell suspension was passed through 70 µM cell strainer (BD-Falcon, USA). Cells were cultured in DMEM-HG with 10% fetal bovine serum (FBS, Gibco-Invitrogen), 100 U/mL penicillin, and 100 µg/ml streptomycin at 37°C in 5% CO_2 incubator and the medium was changed twice a week. Cells cultured up to at passage 4 (P4) were employed for analyses. All experiments were carried out in triplicates.

Morphology and Viability of hGMSCs

After the isolation, GMSCs were plated at a density of 1×10^5 cells on 35 mm culture dishes. Morphology of GMSCs was observed and photographed by phase-contrast microscopy (Olympus, Japan). Percentage of live cells was calculated at every passage of GMSCs from passage 1 (P1) to passage 4 (P4). Cell viability was evaluated using 0.4% trypan blue (Gibco-Invitrogen). Cells which stained blue was considered as dead cells and transparent cells were counted as live cells using haemocytometer.

Colony-Forming Unit (CFU) Assay

A total of 0.5×10^3 GMSCs were seeded on to 35 mm dish and cultured for 14 days with a change of fresh media every third day. GMSCs were assessed for the ability of colony formation by crystal violet (0.5% w/v) staining. Colonies with > 50 cells were examined and photographed.

Proliferation and Population Doubling Time (PDT) Assay

Cell proliferation assay of GMSCs was performed at passage 3 (P3) by seeding 1×10^4 cells on a 12-well plate on day 0 and counting the cells at days 3, 6, 9 and 12 by trypsinization using a hemocytometer under phase-contrast microscope. In order to determine the PDT, the formula employed was: $PDT = t\ (\log 2) / (\log Nt - \log No)$, where t represents culture time, and No and Nt are the cell numbers before and after seeding, respectively.

Expression of Cell Surface Markers by Flow Cytometry

Cell phenotypes were assessed by the expression of cell surface markers, such as CD29, CD44, CD73, CD90, (positive markers) and CD34 and CD45 (negative markers) using flow cytometer (BD FACSCalibur, Becton Dickinson, USA). The cells at a density of 0.5×10^6 were

used for each marker. Primary antibodies (1:100 dilution, Biolegend or eBioscience, USA) were added and incubated at 37°C for 1 hour. Fluorescein isothiocyanate (FITC)-conjugated IgG (eBioscience) was used as a secondary antibody and incubated for 1 hour at room temperature in dark. A total of 10,000 cells were acquired for each sample along with isotype-matched control and analysed with Cell Quest software (Becton Dickinson).

Adipogenic Differentiation of hGMSCs

For adipogenic differentiation, hGMSCs were seeded at a density of 2×10^4 cells/well in 12-well plate. After attaining 70% confluency, cells were cultured in induction media consisting of 0.5 mM 3-isobutyl-1-methylxanthine (Sigma-Aldrich), 1 µM dexamethasone (Sigma-Aldrich), 0.1 mM indomethacin (Sigma-Aldrich), 10 µg/ml insulin (Sigma-Aldrich), and 10% FBS (Gibco). Medium changes were carried out twice weekly and adipogenesis was assessed on day 21 of induction. The presence of lipid droplets was evaluated by staining with 0.5% (w/v) Oil Red O (Sigma-Aldrich) solution prepared in 60% isopropanol (Sigma-Aldrich). The presence of oil droplets in culture was imaged with a phase-contrast microscope (Olympus).

Preparation of hDTM

The hDTM was prepared using a previously developed method with minor modifications (Gomes *et al.*, 2001). Briefly, non-carious teeth of patients whose teeth were extracted for clinical reasons were thoroughly washed with 0.2% chlorhexidine, 0.9% saline and graded ethanol. Then, crown part of the teeth were pulverised with dry ice for 20 seconds and air-dried aseptically. The powder sieved through 300 µM was subjected to demineralisation process using 0.6 N hydrochloric acid in the ratio of 10 gm of teeth powder to 100 mL of acid solution overnight. After demineralisation, matrix was washed with distilled water to get rid of all the acid traces until the pH of the washed solution reached the neutral pH (6.5 to 7.0). The matrix was again washed with pre-cooled milli-Q water (6.5–7.0), and dehydrated with graded ethanol twice for 15 minutes followed by diethyl ether wash. Finally, the DTM was air-dried aseptically and stored at –80°C for further use.

Interaction of hGMSCs with hDTM

In order to determine the biocompatibility of hDTM with GMSCs, cells were cultured with hDTM and analysed for the morphology by phase-contrast microscope and viability by 0.4% trypan blue exclusion test. Viability was assessed from day 1 to day 4 of culture by counting the cells at 24 hours interval using a hemocytometer.

Osteogenic Differentiation of hGMSCs

The hGMSCs were seeded at the density of 2×10^4 cells/well in 12-well plate and cultured in osteogenic induction (OI) medium containing 10 mmol/L β-glycerophosphate (Sigma-Aldrich), 0.2 mmol/L ascorbate-2-phosphate (Sigma-Aldrich), 100 nmol/L dexamethasone (Sigma-Aldrich), and 10% FBS for 3 weeks. After osteogenic differentiation, cells were fixed in 10% neutral buffered formalin (Sigma-Aldrich) for 15 minutes and were stained with Alizarin red S (Sigma-Aldrich) for confirming the deposition of mineralised calcium. The following four groups were subjected for in vitro differentiation of GMSCs with hDTM.

A. hGMSCs + Culture medium (Control): 2×10^4 cells/well were seeded onto 12-well plate, and cultured up to 21 days without addition of the osteoinduction supplements and the medium was changed twice a week.

B. hGMSCs + OI: 2×10^4 cells/well were seeded onto 12-well plate, and cultured up to 21 days with addition of the osteoinduction supplements and the medium was changed twice a week.

C. hGMSCs + hDTM: 2×10^4 cells/well were seeded along with 0.1 mg of hDTM onto 12-well plate, and cultured up to 14 days without addition of the osteoinduction supplements and the medium was changed twice a week.

D. hGMSCs + hDTM + OI: 2×10^4 cells/well were seeded along with 0.1 mg of hDTM onto 12-well plate, and cultured up to 14 days with the addition of the osteoinduction supplements and the medium was changed twice a week.

Statistical Analysis

The data are expressed as the mean ± standard deviation (SD). Analysis of variance (ANOVA) was performed by GraphPad Prism software (GraphPad, CA, USA) with Tukey's post-hoc test. The level of significance was considered when $p < 0.05$.

RESULTS

Morphology, Colony-Forming Units and Growth Kinetics

Plastic adherent GMSCs exhibited a characteristic fibroblast-like morphology at 10–12 days in primary culture (Fig. 1A). GMSCs retained small and homogeneous appearance throughout the culture period. GMSCs showed > 91% viability at each passage from P1 to P4 with highest of 96% at P3 (Fig. 1B). GMSCs demonstrated their propensity to form colonies (CFUs) or clusters when culture expanded at low density (Fig. 1C). Further, GMSCs showed greater proliferative activity from day 6 of culture and reached a peak on day 9 with significant ($p < 0.05$) increase in numbers (Fig. 1D). The values of PDT varied between 39.34 hours and 62.59 hours, and was increased as the day of culture prolonged.

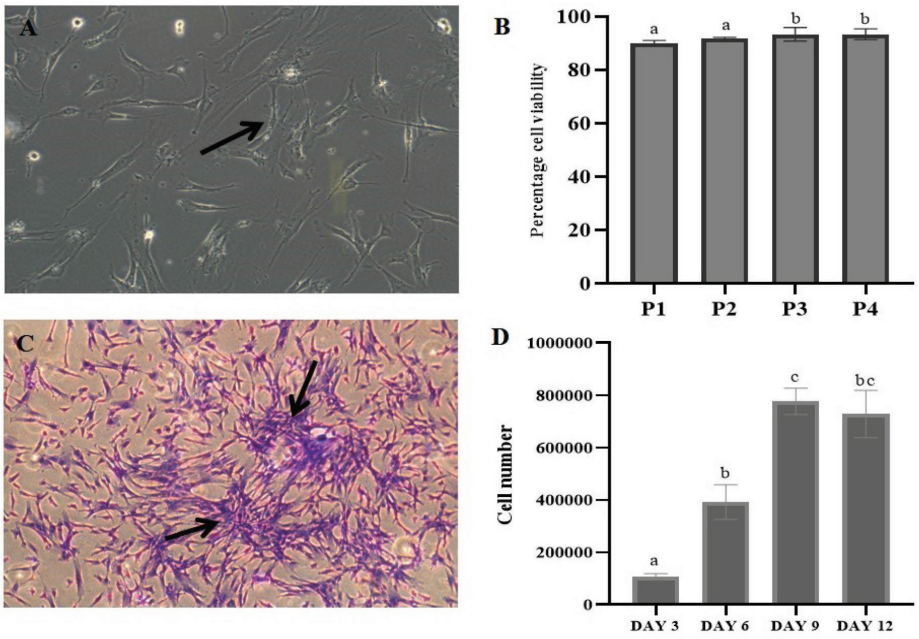

Fig. 1 Characterisation of hGMSCs: (A) Plastic adherent cells exhibited a characteristic fibroblast-like morphology (arrow) at 10–12 days of culture; (B) GMSCs showed > 91% viability at each passage and the highest viability of 96% was observed at P3. Mean ± SD values are presented from triplicates performed at each passage; (C) A microscopic image indicating the colony-forming ability of hGMSCs (arrows) stained by Crystal violet; (D) Cell proliferation assay was performed by counting the cells at days 3, 6, 9 and 12 using a hemocytometer. Values are represented as means ± SD of triplicates at each time interval. Superscripts a, b and c indicate significance at $p < 0.05$. Images: 4×.

Expression of Cell Surface Markers

GMSCs were positive for MSCs-associated markers, such as CD29, CD44, CD73 and CD90 with expression levels ranging from 60.93% to 80.90% (Fig. 2). In contrast, GMSCs were negative for hematopoietic markers CD34 and CD45 with expression levels of < 1% (Fig. 2). The expression level of the selected markers was stable during the culture expansion.

Adipogenic Differentiation

Differentiation towards adipogenic phenotypes was assessed by cytochemical staining (Fig. 3). Lipid droplets appeared in GMSCs of monolayer cultures treated with adipogenic induction medium (Fig. 3B) and was absent when compared to the untreated control cultures (Fig. 3A). Oil Red O staining of day 21 cultures confirmed the presence of neutral lipid positive vacuoles.

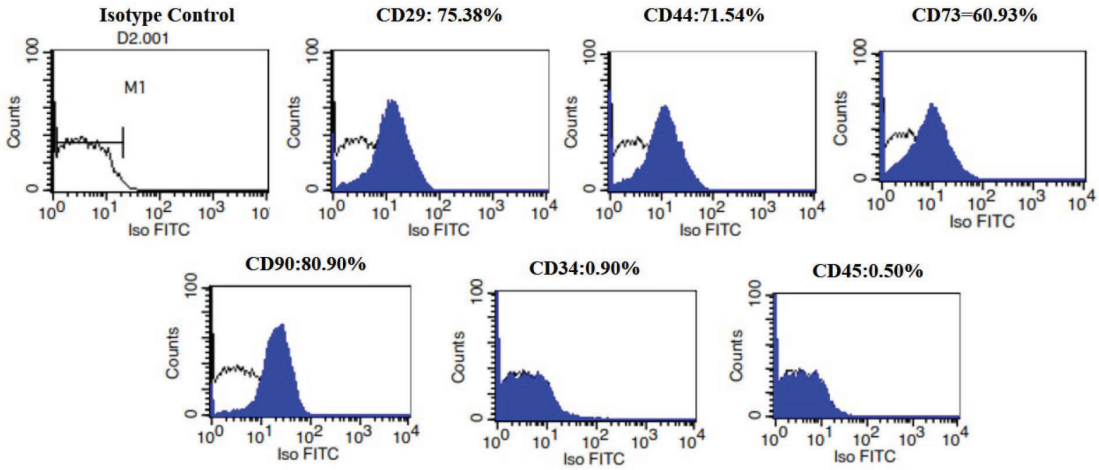

Fig. 2 Flow cytometry analysis of cell surface marker expression in hGMSCs. Representative images indicating the levels of marker expression are shown. hGMSCs expressed CD29, CD44, CD73 and CD90 markers, but were negative for CD34 and CD45. Isotype control was analysed in parallel. In merged images, dark-lined histograms indicate the isotype-control and blue-filled histograms show the reactivity with indicated antibody.

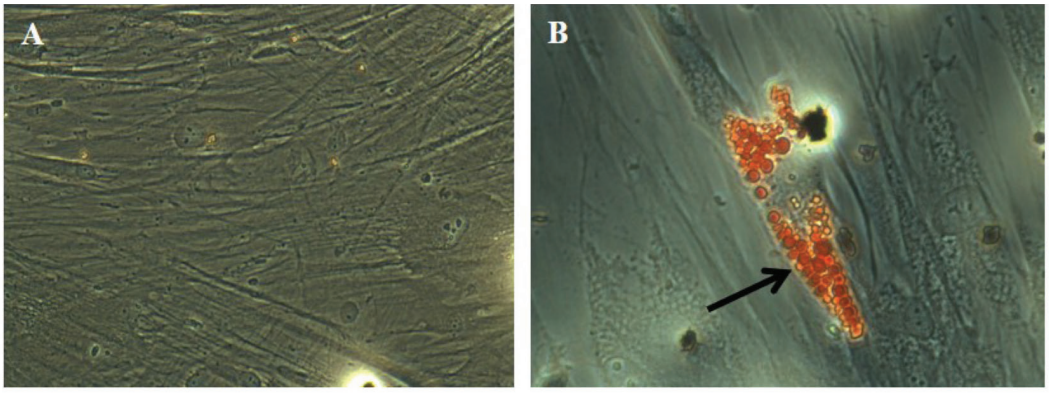

Fig. 3 Adipogenic differentiation of hGMSCs: (A) Cells in non-inductive medium maintained a fibroblastic morphology; (B) Oil Red O staining of day 21 cultures confirmed the presence of neutral lipid positive vacuoles (arrow) in induced hGMSCs. Image: 20×

Biocompatibility and Viability of hGMSCs Cultured with hDTM

GMSCs were cultured in hDTM for 96 hours and assessed for biocompatibility. No noticeable adverse changes in morphological features were observed in GMSCs when cultured with hDTM (Fig. 4A). Further, the cells were found to be proliferative and increased their number with higher viability in the presence of hDTM (Fig. 4B). The viability values ranged from 87% to 93%, and indicated that hDTM was not cytotoxic to GMSCs.

Osteogenic Differentiation of GMSCs Cultured with hDTM in the Presence or Absence of Osteogenic Inducers

GMSCs were seeded along with hDTM and cultured up to 14 days with the presence or absence of the osteogenic induction (OI) supplements. Cells in control exhibited a fibroblastic morphology during the culture period (Fig. 5). Mineralised calcium matrix was observed on the monolayer cultures GMSCs with hDTM when compared to their absence in untreated cultures of GMSCs alone. Alizarin red S staining of day 14 cultures clearly demonstrated the accumulation of mineralised matrix and confirmed the osteogenic potential of GMSCs in the presence of hDTM. Further, enhanced differentiation of GMSCs into osteocytes was clearly observed when the cells were cultured along with hDTM and OI supplements (Fig. 5).

DISCUSSION

The basic characterisation of GMSCs has demonstrated that they possess all the phenotypical and biological features specified under the stem cell criteria, and hence can be used in bone tissue regeneration (Mitrano *et al.*, 2010). In the present study, GMSCs were successfully isolated from healthy human gingiva. These plastic adherent cells showed a greater proliferative activity and viability after attaining characteristic fibroblast-like morphology as documented previously (Tomar *et al.*, 2010; Mitrano *et al.*, 2010; Tang *et al.*, 2011; Jauregui *et al.*, 2018; Al-Qadhi *et al.*, 2020; Subba *et al.*, 2022). Earlier study compared the morphological characteristics of GMSCs and BMSCs, and found that GMSCs are uniformly homogenous in primary cultures

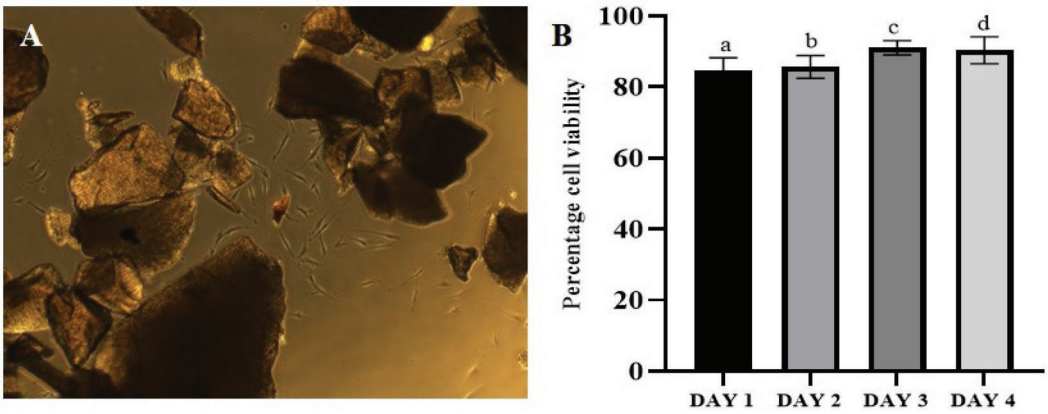

Fig. 4 Biocompatibility and viability of hGMSCs cultured with hDTM: (A) No noticeable adverse changes in morphology were observed in hGMSCs when cultured with hDTM; (B) The viability of hGMSCs ranged from 87% to 93% and these values indicated that hDTM was not cytotoxic to GMSCs. Values are represented as means ± SD of triplicates at each time interval. Superscripts a, b, c and d indicate significance at $p < 0.05$. Image: 4×.

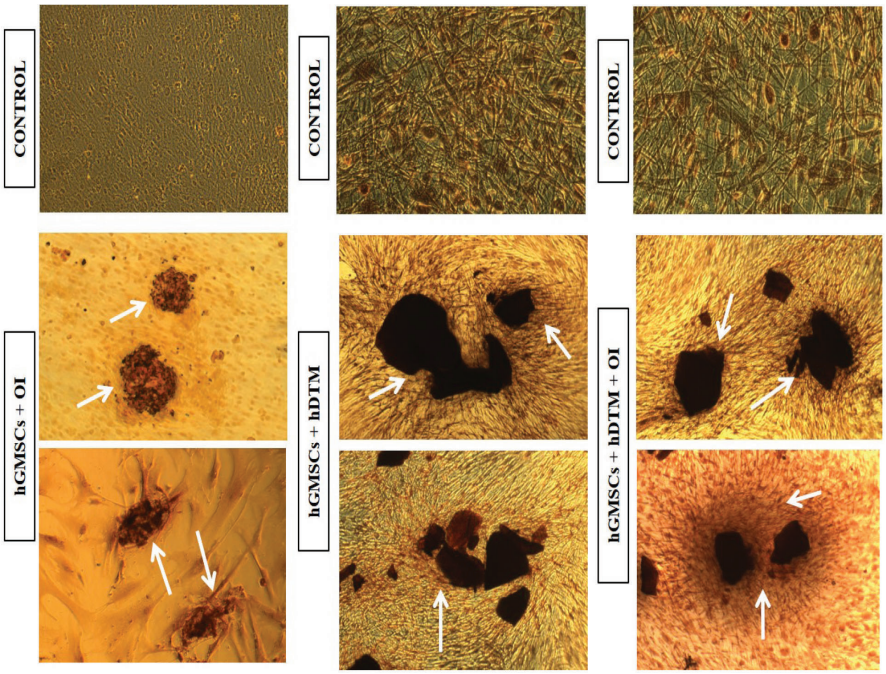

Fig. 5 Osteogenic differentiation potential of hGMSCs. Cells in non-inductive medium (control) exhibited a fibroblastic morphology during the culture period of 21 days. Mineralisation of calcium nodules appeared in hGMSCs cultured with osteogenic induction (OI) medium (arrows), hDTM (arrows), and hDTM along with OI (arrows). Alizarin red S staining confirmed the presence of mineralized matrix with varied intensities. Images: 4× and 10×.

and maintained uniformly spindle-shaped fibroblast-like even in late passages (Tomar *et al.*, 2010). Further, the CFU assay demonstrated the formation of colonies by GMSCs and this observation corresponded generally to earlier reports (Du *et al.*, 2016; Santamaría *et al.*, 2017). The PDT in this study was found to increase as the day of culture prolonged. In the comparative studies reported previously, it was found that GMSCs have a higher proliferation rate and lesser PDT when compared to DPSCs, PDLSCs and BMSCs thus making expansion of GMSCs easier ex vivo (Tomar *et al.*, 2010; Tang *et al.*, 2011; Gao *et al.*, 2014; Al-Qadhi *et al.*, 2020). The DPSCs and GMSCs have higher proliferative and CFU ability which may be due to the fact that the healing and regenerative capacity of these tissues is higher than that of the periodontal ligament (Gao *et al.*, 2014). Collectively, these growth kinetics and potency properties of GMSCs can be advantageous in terms of easy in vitro propagation and greater in vivo tissue regeneration.

In this study, GMSCs expressed CD29, CD44, CD73 and CD90, which are the surface markers for MSCs and negative for CD34 and CD45, which are the leucocyte precursor markers. Although all the types of MSCs largely possess these markers, the amount of expression might be different in GMSCs (Ge *et al.*, 2012; Kim *et al.*, 2021; Kumar *et al.*, 2021a; Subba *et al.*, 2022). Among the markers, the expression of CD90 was higher in GMSCs when compared to PDLSCs and DPSCs (Gao *et al.*, 2014), whereas CD29 marker was found to be higher when compared to dermal-fibroblast-derived stem cells (Fournier *et al.*, 2010). The differences in the expression of these markers are likely due to their anatomical origin and the uniqueness of GMSCs (Kim *et al.*, 2021; Subba *et al.*, 2022).

In the present study, GMSCs were able to induce into adipogenic and osteogenic differentiation with lineage-specific induction media. These results were consistent with the previous studies that demonstrated presence of lipid-rich vacuoles confirmed by Oil Red O staining and deposition of mineralized calcium confirmed by Alizarin red staining (Mitrano *et al.*, 2010; Tomar *et al.*, 2010; Santamaría *et al.*, 2017; Kumar *et al.*, 2021a; Subba *et al.*, 2022).

The tooth-derived material as a scaffold has attracted attention in recent times due to the ease of availability of teeth (Bono *et al.*, 2017; Um *et al.*, 2017; Kabir *et al.*, 2021; Kumar *et al.*, 2021b). The teeth and the bone are similar histologically and embryologically as both are derived from the neural crest cells (Kim *et al.*, 2010). Human DDM is considered one of the most acid-soluble scaffolds, which contains collagen matrix and osteoinductive growth factors and a mineral phase, which makes it almost an ideal bone substitute. In this study, we have used hDTM as a scaffold for GMSCs and assessed in vitro on their biocompatibility and osteogenic potential (Liu *et al.*, 2016).

In the present study, hDTM showed no adverse impact on the morphology and viability of GMSCs when cultured for 96 hours. Earlier study on the biocompatibility and interaction of DDM with DPSCs demonstrated an increased adhesion, migration capability and osteogenic and odontoblastic differentiation of DPSCs (Liu *et al.*, 2016). As DDM granules contain growth factors, such as BMP-2, transforming growth factor-beta 1 (TGF-β1) and basic fibroblast growth factor (bFGF), they offer nutrients and assist in adherence and migration of DPSCs (Ge *et al.*, 2012). Further, the demineralisation process increased the bioavailability of BMP-2 (Bono *et al.*, 2017). In a recent study, DTM cultured with DPSCs indicated their biocompatible features with unaltered morphology and enhanced the proliferation ability and cell viability (Kumar *et al.*, 2021b). The higher survival of DPSCs indicated the minimal role of DTM in causing toxicity.

In this study, GMSCs were seeded along with hDTM and cultured up to 14 days in the absence of OI supplements. The cultures showed a low level of mineralized matrix formation, indicating the osteogenic potential of GMSCs in the presence of hDTM. Similar results were observed by Kang *et al.* (2017), wherein they compared the osteogenic differentiation of hydroxyapatite-tricalcium phosphates (HA-TCP) and DDM on DPSCs and found that bone induction using DDM was by osteoinduction due to the increased expression levels of alkaline phosphatase (ALP), bone sialophosphoprotein (BSP), osteopontin (OPN), dentin sialophosphoprotein (DSPP), and dentin matrix protein (DMP-1) in cells cultured with DDM (Lee *et al.*, 2015). Further, the culture of GMSCs with hDTM in the presence of osteogenic supplements demonstrated the enhanced accumulation of mineralised matrix on day 14 of culture. According to the previously published reports, organic phosphate in β-glycerophosphate is believed to get hydrolyzed by ALP and released inorganic phosphate can promote the deposition of mineral on the tissue culture surface and other materials (Chen *et al.*, 2019). Other results showed that hDTM had substantial influence on DPSCs osteogenic differentiation and mineralisation (Kumar *et al.*, 2021b). In hDTM, the mineral components and antigenic molecules were absent following demineralisation, and this could have supported the osteocytes formation in vitro. Further, these observations are in accordance with previous reports employing demineralised dentin or enamel matrix for bone tissue regeneration (Kim *et al.*, 2010; Wang *et al.*, 2011; Kang *et al.*, 2017; Um *et al.*, 2017; Kabir *et al.*, 2021). Thus, these findings corroborate the current results that the osteogenic differentiation was enhanced when the GMSCs were induced with hDTM in the presence of osteogenic supplements.

CONCLUSION

Isolated GMSCs exhibited MSCs-like properties in terms of morphology, growth kinetics, markers expression and mesenchymal lineage differentiation. The hDTM was observed biocompatible and did not exert any cytotoxic effect when culture expanded with GMSCs. Though the culture of GMSCs with hDTM showed their osteogenic potential, the presence of osteogenic supplements supported the enhanced osteogenesis of GMSCs. Further studies are warranted to explore the potential of clinical grade GMSCs in bone tissue regenerative applications using teeth matrix as a scaffolding material.

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