Original Article

Comparison of Cellular and Differentiation Characteristics of Mesenchymal Stem Cells Derived from Human Gingiva and Periodontal Ligament

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Received : 11-09-21
Revised : 01-12-21
Accepted : 15-12-21
Published : 08-04-22

ABSTRACT

Objectives: Dental tissues possess multipotent stem cells with varying biological properties. The present study was aimed to establish a primary culture of human gingiva-derived mesenchymal stem cells (GMSCs) and periodontal ligament-derived stem cells (PDLSCs) from periodontally healthy subjects and compare their biological characteristics. **Materials and Methods:** Gingival and periodontal ligament (PDL) tissues were collected from extracted premolar teeth of five healthy subjects and primary cultures were established. Basic biological characteristics, such as cell morphology, viability, proliferation capacity, and colony-forming units, and *in vitro* osteogenic and adipogenic differentiation potential were performed at passage 3 of GMSCs and PDLSCs. This was followed by immuno-phenotyping and flow cytometric analysis for identification of positive mesenchymal stem cell (MSC) markers, such as CD73, CD90, and CD105, and negative markers CD45 and CD34. **Statistical Analysis Used:** One-way analysis of variance (ANOVA). **Results:** Primary cultures of GMSCs and PDLSCs were successfully established. Cells exhibited a fibroblast-like morphology with a homogeneous population at passage 3. Cells derived from both tissues were highly viable (>95%), proliferative, and capable of forming colonies. Both cells did not exhibit any noticeable differences in cellular properties. Immunofluorescence and flow cytometric analyses showed positivity for MSC markers, CD73, CD90, and CD105, and negativity for CD34 and CD45. Furthermore, GMSCs and PDLSCs were capable of differentiating *in vitro* into osteocytes as evidenced by Alizarin red-S staining, and adipocytes as demonstrated by oil red O staining. **Conclusions:** The results of the present study indicate that both GMSCs and PDLSCs have similar cellular characteristics and mesenchymal differentiation potential. Therefore, they may serve as an equally potent source of stem cells for use in cell-based periodontal therapies.

Keywords: *Comparative characterization, gingiva, periodontal ligament, stem cells*

Introduction

Periodontitis is a chronic inflammatory disease that is initiated by a specific microorganism or groups of microorganisms. It is characterized by progressive destruction of the supporting structures of the teeth with pocket formation, recession, or both.[1] Periodontitis is prevalent in 11%–15% of the population being affected at some stage in their lives. If left untreated, periodontitis results in eventual

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Quick Response Code:

Website: www.jispcd.org

DOI: 10.4103/jispcd.JISPCD_259_21

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How to cite this article: Subba TA, Varma S, Thomas B, Rao S, Kumar M, Talwar A, *et al.* Comparison of cellular and differentiation characteristics of mesenchymal stem cells derived from human gingiva and periodontal ligament. J Int Soc Prevent Communit Dent 2022;12:235-44.

tooth loss.[1] Thus, proper management and timely treatment are essential. Once the destruction of the periodontium has occurred, it has a limited potential to regenerate. Surgical treatment procedures like open flap debridement are useful for access to defects but heal by repair.[2] Periodontal ligament (PDL) cells have demonstrated the ability to form new attachments by their innate ability to differentiate into periodontal ligament-forming cells, cementoblasts, or osteoblasts. Based on this evidence, guided tissue regeneration (GTR) was developed on the principal that regeneration can occur by selective repopulation of the periodontal defect with progenitor cells of the PDL. Though GTR has shown considerable clinical success by means of reduction in probing depth and bone fill when used alone or with bone replacement grafts, the regeneration of the supporting structures of the teeth has been unpredictable.[2,3] This unpredictability may be attributed to the complexity of the periodontal structures and the limited availability of progenitor cells at the periodontal defect site.[2,3]

To overcome the limitations of conventional periodontal regeneration procedures, the application of cell-based therapy has attracted a lot of attention and interest in the past decade.[4] The ability of mesenchymal stem cells (MSCs) to self-perpetuate and their multilineage differentiation potential makes these cells ideal for regenerative therapy.[4] Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) in 2006 defined MSCs based on their ability to adhere to plastic, express specific markers, and differentiate into different cell lines.[4] Among the MSCs, bone marrow-derived MSCs have been utilized for various regeneration therapies. However, the disadvantage of these bone marrow MSCs is that it is a traumatic and painful procedure to harvest these cells and the culture studies show that they are seen in less numbers. Therefore, MSCs from dental tissues, such as the periodontal ligament, gingiva, dental follicles, dental pulp, apical papilla, and human exfoliated deciduous teeth are being studied for harvesting because of the easy access and more/comparative number of stem cells harvested.[5]

Periodontal ligament stem cells (PDLSCs) have demonstrated their periodontal regenerative potential *in vivo,* when appropriately stimulated, thus making them an ideal candidate for use in periodontal therapy. PDLSCs expressed MSC markers and differentiated into cementoblast-like cells, adipocytes, and collagen-forming cells.[6,7] Studies suggested that PDLSCs induced cementum-like layer or at least the implantation of cells showed beneficial results in the defect sites in many animal models.[8] Thus, sufficient evidence from preclinical animal studies warranted moving to human studies that have, to date, shown that PDLSCs are safe and feasible to be used for therapeutic tissue regeneration.[9,10] However, it is necessary to extract the tooth to harvest the tissue, which is also less in its source. Due to these practical disadvantages, the gingiva has been considered as an alternative source of MSCs.[11]

Gingiva is easily accessible as a byproduct of certain routine periodontal surgical procedures. Following the excision of gingival tissue, the subsequent wound healing is fast and uneventful. In addition, they demonstrate fetal-like cell healing properties. Gingiva-derived mesenchymal stem cells (GMSCs) have shown rapid expansion of cells, the presence of MSC markers, and differentiated into osteoblast, adipocyte, and chondrocyte lineages.[11,12] Animal studies of transplanted GMSCs in induced defects have found that the results are encouraging for further use of these GMSCs in therapeutic tissue regeneration.[13-15] Current evidence suggests that GMSCs demonstrate a stable phenotype in long-term cultures and may be superior to bone marrow-derived MSCs for cell-based regenerative therapy.[11-15]

Limited data are available to support the use of GMSCs as alternative cell types to PDLSCs for periodontal regeneration, thus necessitating further research.[15,16] A study by Nugraha *et al.* in Wistar rats shows that GMSCs are multipotent and may be advantageous for tissue engineering and regenerative therapy.[17] Therefore, this study aimed to isolate and establish a primary culture of human GMSCs and PDLSCs from the same periodontally healthy subjects and compare their basic biological properties and assess their *in vitro* differentiation potential into osteocytes and adipocytes.

Materials and Methods

Sample population

Five systemically healthy female patients (aged 14–27 years; mean age: 20.5 years) requiring extraction of periodontally healthy premolar teeth for orthodontic reasons were enrolled for the study after obtaining written informed consent from the patient or legal guardian. Only female patients happened to be chosen as during the period of sample collection, their orthodontic treatment plans included extraction of premolars, and one male sample taken was not included as it showed error during cell growth. The study was approved by the Ethics Committee of A. B. Shetty Memorial Institute of Dental Sciences

(Cert No.ABSM/EC87/2015), Nitte (Deemed to be University), and the Nitte University Institutional Committee for Stem Cell Research (IC-SCR No.NU/ICSCR/2016–17/002A/P1).

Tissue sample collection

The extracted premolars were used for tissue biopsy and PDL cell isolation. Gingival tissues of size 3 mm × 2 mm × 2 mm surrounding the tooth sockets were collected immediately after tooth extraction. The gingival tissue sample was taken under the same anesthesia and the healing of the socket was not compromised. PDL tissue was separated from the middle third of the root using a fresh surgical scalpel after rinsing the tooth in saline. Individual differences were eliminated by only including samples from individuals in whom both PDLSCs and GMSCs could be isolated from the same tooth ($n = 5$). The gingival and PDL tissue samples were immediately placed in separate sterile vials containing Dulbecco's phosphate-buffered saline (D-PBS) (Gibco-Invitrogen, Life Technologies, Grand Island, NY, USA) with antibiotics-penicillin and streptomycin (Gibco-Invitrogen).

Establishment of primary cell culture

The primary culture was established using previously established protocols.[5,6] Briefly, tissue was minced into <1 mm length using a sterile surgical blade and scalpel. The tissue pieces were incubated in 0.1% collagenase type IV enzyme (Gibco-Invitrogen) for 1 h and then the enzyme digested tissue sample was centrifuged at 1000 rpm for 5 min and the supernatant was discarded. Later, 4–5 tissue explants were placed in culture dish overnight, with a minimum amount of Dulbecco's modified Eagle's medium (DMEM)-High glucose (Gibco-Invitrogen) with 20% fetal bovine serum (FBS, (Gibco-Invitrogen), 100 U/mL penicillin and 100 µg/mL streptomycin solution (Gibco-Invitrogen). The culture was continued till the cells reached 60%–70% confluence.

Cell viability

The percentage of live cells was calculated at every passage (P) of PDLSCS and GMSCS from P-1 to P-5. Cell viability was assessed using 0.4% trypan blue (Gibco-Invitrogen) staining in a hemocytometer. Cells that stained blue were considered dead cells and transparent cells were counted as live cells.

Colony-forming unit assay

A colony-forming unit (CFU) assay was carried out for both PDLSCs and GMSCs following established methodology.[7] Cells were stained with Crystal violet for 20 min at room temperature. The excessive stain was washed using deionized water and observed under a phase-contrast microscope (Olympus, Tokyo, Japan) for colony formation.

Proliferation analysis and population doubling time

Population doubling time and proliferation analysis of both the cells were assessed at P-3. Five thousand cells were plated in 12-well plates and every three days cell counting was carried out using a hemocytometer for 12 days. Total four readings were obtained with increasing cell numbers. Population doubling time (PDT) was calculated as, PDT = t (log2)/(log Nt-log No), where t represents culture time, and No and Nt are the cell numbers before and after seeding, respectively.

Immunofluorescence analysis

Expression of cell surface-specific markers was analyzed by immunofluorescence staining. GMSCs and PDLSCs were cultured on four-well cell imaging chamber slides (Eppendorf, Germany) until they reached 80% confluence. Cells were washed twice with D-PBS before being fixed in 3.7% paraformaldehyde (Sigma-Aldrich, USA) for 30 min. Following three washes with D-PBS, cells were blocked in D-PBS with 2% bovine serum albumin (BSA) (Sigma-Aldrich) for 1 h. Then the cells were incubated overnight at 4°C with the following monoclonal or polyclonal primary antibodies: anti-human CD73 (Biolegend, USA, 1:100), anti-human CD90 (Thy-1, E-bioscience, USA, 1:100), anti-mouse CD105 (Biolegend, 1:50), anti-human CD34 (Biolegend, 1:100), and anti-human CD45 (E-bioscience, 1:100). After being rinsed three times with D-PBS, the cells were incubated with fluorescein isothiocyanate (FITC) conjugated secondary antibodies (goat anti-mouse IgG, E-bioscience, USA, mouse IgGk BP-FITC, Santa Cruz Biotechnology, USA, 1:100) for 45 min at 37°C and again rinsed three times with D-PBS. Nucleus was counterstained with 1 µg/mL propidium iodide (PI) (Gibco-Life Technologies, USA) or 1 µg/mL 4',6-diamidino-2-phenylindole (DAPI) (Cytocell Ltd., UK) for 5 min at room temperature, and the slides were mounted with Pro Long Diamond Antifade Mountant (Molecular Probes, USA). Images were observed under a fluorescence microscope (Olympus).

Cell surface antigen profile by flow cytometry analysis

GMSCs and PDLSCs were characterized for the presence of mesenchymal markers (CD73, CD90, and CD105) and absence of CD34 and CD45 using flow cytometry (BD FACS Calibur, Becton Dickinson, USA). Both types of cells at approximately 80% confluence were fixed with 3.7% paraformaldehyde for 30 min. Alexa fluor-488 conjugated anti-mouse CD105 (Biolegend, 1:50) was labeled directly at 37°C for 1 h, and unconjugated CD73 (Biolegend, 1:100),

CD90 (E-bioscience, 1:100), CD34 (Biolegend, 1:100), and CD45 (E-bioscience, 1:100) were incubated for 2 h at 37°C. Following washes with cell staining buffer (Biolegend, USA), FITC-conjugated anti-mouse IgG (E-bioscience, 1:100) used as a secondary antibody was labeled for 1 h at room temperature. The standard was established by isotype-matched control (E-bioscience, USA). A total of 10,000 FITC-labelled cells were acquired and analyzed by a BD FACS Calibur with Cell Quest software (Becton Dickinson).

Osteogenic and adipogenic differentiation

Osteogenic and adipogenic differentiation was induced using a previously established protocol.[5,6] The P-3 cells were treated with osteogenic and adipogenic medium for three weeks. Differentiation to osteoblasts was demonstrated by staining with alizarin red and staining with 0.5% w/v oil Red O solution demonstrated adipocyte differentiation. PBS washed cells were fixed in 3.7% formaldehyde before being stained and imaged (Olympus).

Statistical analysis

The statistical analysis for data was performed with one-way analysis of variance (ANOVA) using Prism version 8.0 software (GraphPad, CA, USA). Data are expressed as mean ± standard deviation (SD). *P* values less than 0.05 indicated statistical significance.

Results

Primary cell culture establishment and morphology

Following the initiation of primary culture, tissue fragments of gingiva and PDL adhered onto the plastic culture dish and the migration of small, round, and spindle-shaped cells was observed after 24–48 h [Figure 1A and C]. During the next five days of culture, cells released from gingival tissue explants started exhibiting fibroblast-like morphology [Figure 1B]. Spindle-shaped cells grew out from the attached gingival and PDL pieces reached 90% confluency at 14–16 days in the culture plates. After two weeks of primary culture, confluent PDLSCs were trypsinized to obtain monocellular suspension for subculture [Figure 1D].

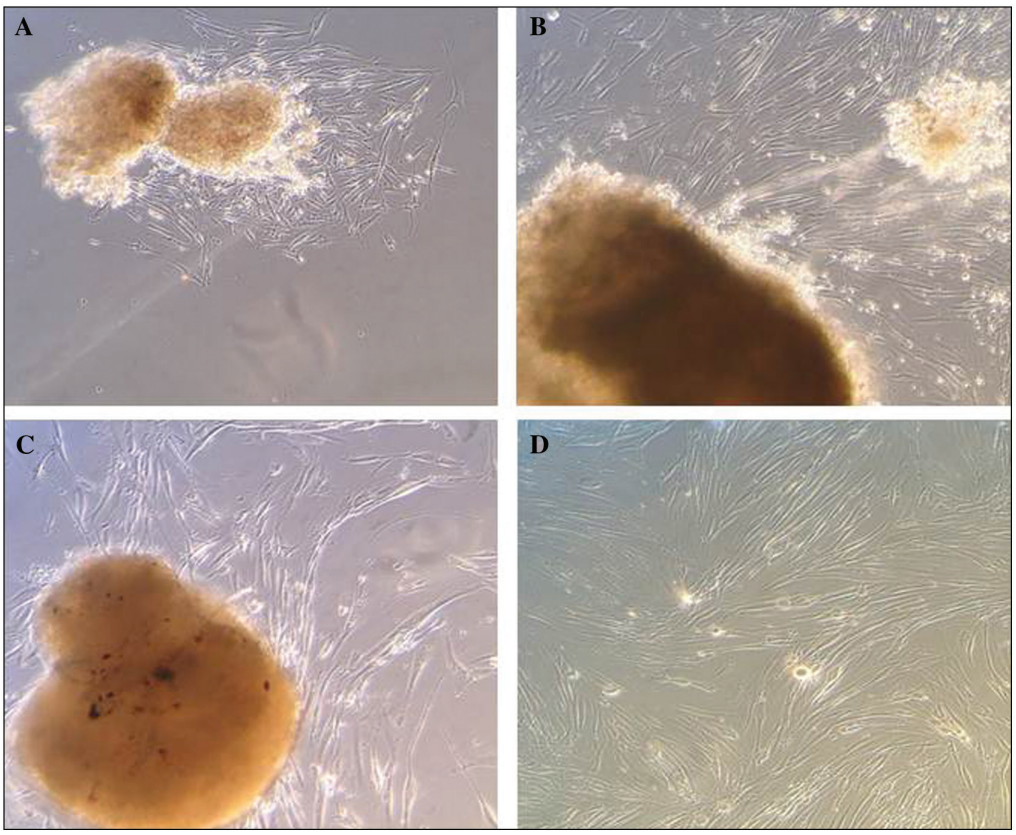

Figure 1: Establishment of primary culture and morphological features of GMSCs and PDLSCs. (A and C) After the initiation of primary culture, tissue fragments adhered onto the plastic culture dish and the migration of round and spindle-shaped cells was observed after 24–48 h. (B) During the next five days of culture, cells released from tissue explants exhibited fibroblast-like morphology. (D) After two weeks of primary culture, cells reached near confluence status (×100)

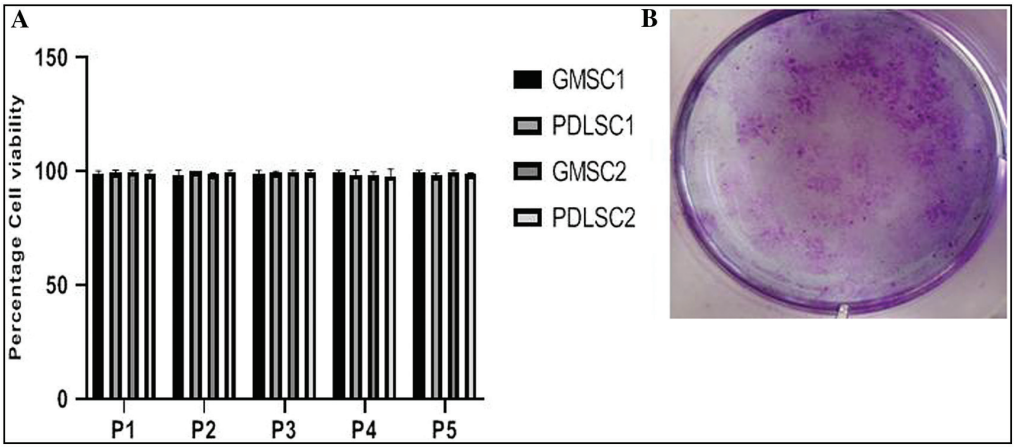

Figure 2: Viability assay and colony forming ability of GMSCs and PDLSCs. (A) Viability assay was performed by trypan-blue exclusion method from passage 1–5 using a hemocytometer. Values are represented as means ± standard deviation (SD) of triplicates at each passage. Cells from all the passages of GMSCs and PDLSCs exhibited >97% viability and no significant difference (P>0.05) was observed between passages as well as cell lines. (B). Macroscopic image of colony-forming ability of GMSCs at 15 days of culture. Cells were stained with Crystal violet

Viability and colony-forming unit assay

The viability of GMSCs and PDLSCs was assessed using 0.4% trypan blue staining and counting of cells in a hemocytometer. The results of the viability assay showed that cells from all the passages (P1–P5) exhibited >97% viability [Figure 2A]. There was no significant difference (P > 0.05) observed between the passages as well as GMSCs and PDLSCs. When GMSCs and PDLSCs were plated at a relatively low density (50 cells/cm^2), GMSCs alone formed CFUs during 15 days of culture [Figure 2B]. However, PDLSCs were unable to form colonies.

Proliferation assay and population doubling time

The results of proliferation assay and PDT of GMSCs and PDLSCs are presented in [Figure 3A and B]. Cell proliferation assay was performed by counting the cells at Days 0, 3, 6, 9, and 12 using a hemocytometer. Both the cell lines showed a significant (P < 0.05) increase in cell number from Day 6 onwards, even though the proliferation rate was slightly slower between Day 0 and Day 3. There was a significant (P < 0.05) difference in PDT of GMSC2 when compared with PDLSC1 and two cell lines. PDT of GMSCs was observed to be 48.8 h and 66.1 h for Sample 1 and Sample 2, respectively. Whereas PDLSCs showed a PDT of 41.7 h and 42.0 h for Sample 1 and Sample 2, respectively. PDLSCs showed shorter PDT when compared with GMSCs.

Analysis of cell surface markers

The study examined the cell surface antigen expression of markers by immunofluorescence and flow cytometry analysis. Results of immunofluorescence assay showed that GMSCs [Figure 4A–F] and PDLSCs [Figure 4F–L] were strongly positive for CD73 and CD90

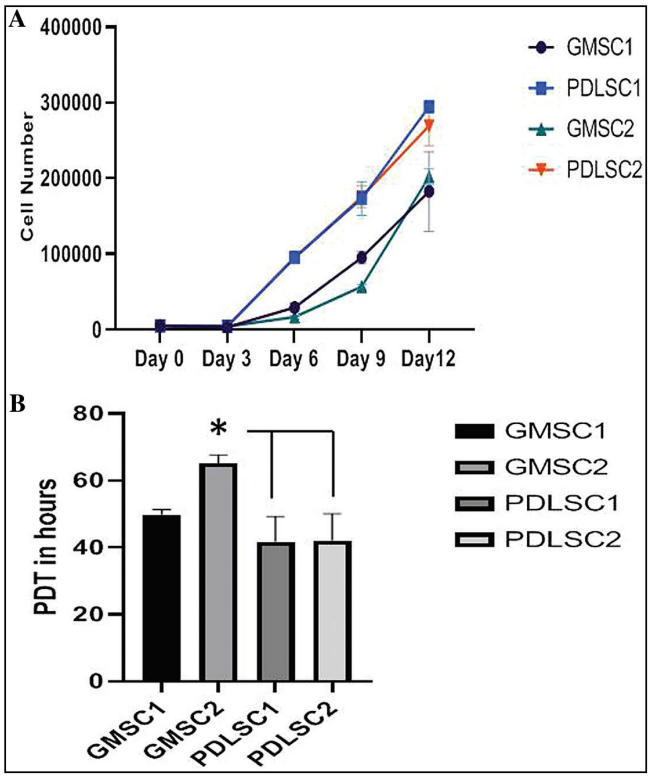

Figure 3: Proliferation assay and population doubling time (PDT) of GMSCs and PDLSCs. (A) Cell proliferation assay was performed by counting the cells at Days 0, 3, 6, 9, and 12 using a hemocytometer. Values are represented as means ± standard deviation (SD) of triplicates at each time point. (B) PDT was calculated using a standard formula. Differences in PDT between the cell lines were analyzed by Sidak's multiple comparison test. P<0.05, indicates significant difference

markers, but the CD105 was weakly expressed. In contrast, the expressions of CD34 and CD45 in both cells were negative. These observations were

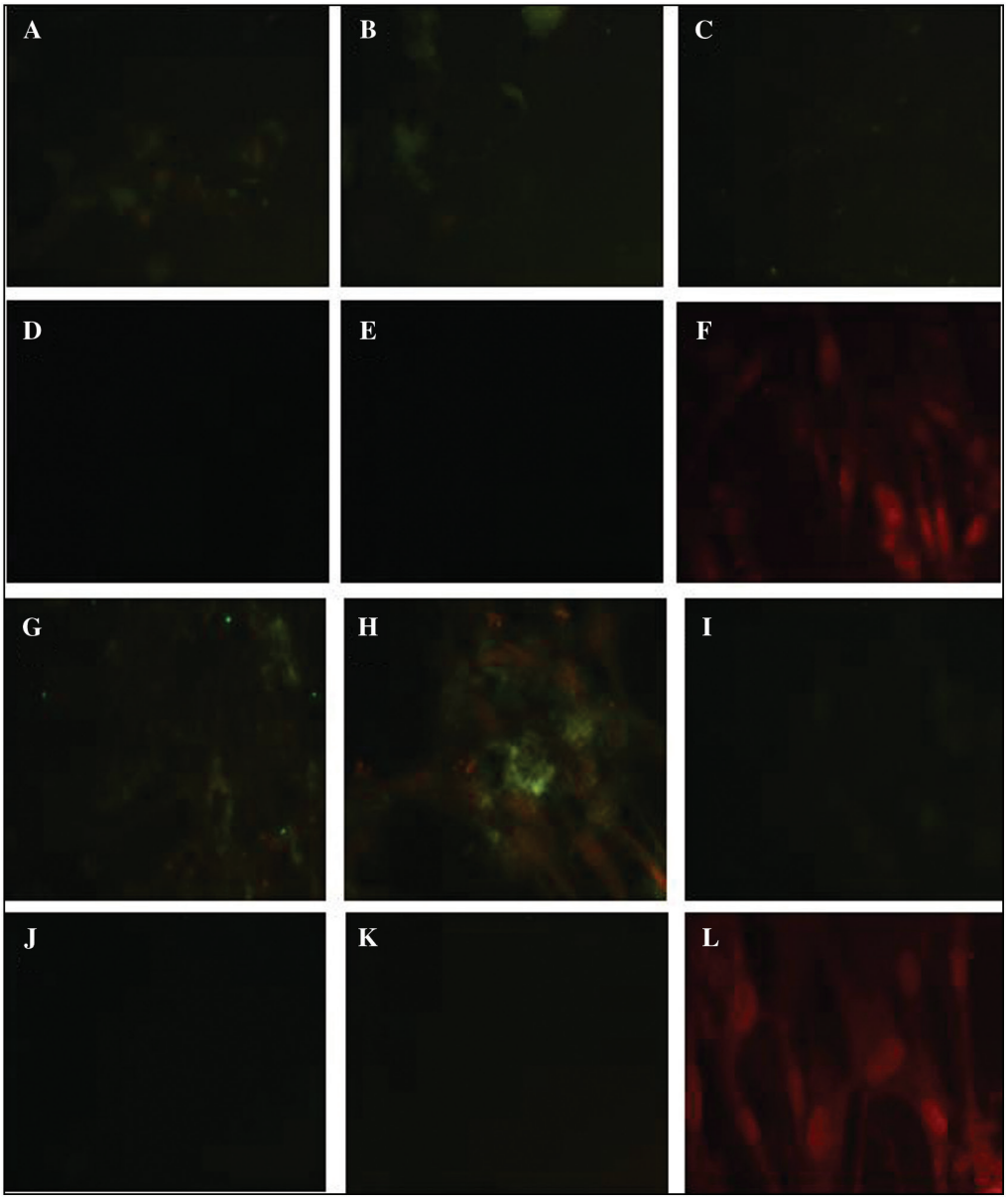

Figure 4: Expression of stem cell markers in GMSCs (A–F) and PDLSCs (G–L) by immunofluorescence analysis. Membrane localization of cell surface antigens reveals immunoreactivity for (A and G) CD73 (FITC, positive), (B and H) CD90 (FITC, Thy-1, positive), and (C and I) CD105 (Alexa Fluor, endoglin, weakly positive). Both cells were negative for (D and J) CD34 (FITC, negative) and (E and K) CD45 (FITC, negative). (F and L) Representative images showing the staining of nucleus by propidium iodide (PI) in GMSCs and PDLSCs, respectively (×20 and ×40)

supported by flow cytometry analysis, as GMSCs [Figure 5A] and PDLSCs [Figure 5B] were positive for CD73 (83.24% and 53.68%, respectively) and CD90 (92.88% and 54.16%, respectively). For CD105 marker, both GMSCs and PDLSCs exhibited relatively low positivity (10.01% and 10.54%, respectively). On the other hand, both cell cultures were negative for the leucocyte precursor markers CD34 and CD45, which suggests the stromal origin of the cells and the absence of hematopoietic precursor cells. GMSCs showed very low or almost negative expression of CD34 (2.38%) and CD45 (2.12%). Similarly, PDLSCs too exhibited a low expression of markers, CD34 (1.79%) and CD45 (1.46%).

Osteogenic and adipogenic differentiation

The results of osteogenic and adipogenic differentiation potential of GMSCs and PDLSCs are presented in [Figure 6]. Both the cells could result in the calcium deposition and mineralization of nodules by osteocytes after three weeks of induction, as demonstrated by Alizarin red-S staining [Figure 6B and D]. Furthermore, the formation of intracellular lipid droplets in adipocytes

was observed in GMSCs and PDLSCS when they were cultured in adipogenic induction media for three weeks. The presence of fat globules was confirmed by oil red-O staining [Figure 6F and H]. However, cells cultured in noninduction medium (control) displayed fibroblast-like features only [Figure 6A and C and E and G].

Discussion

This study was conducted as an *in vitro* research to effectively compare the basic cellular and differentiation characteristics of the mesenchymal stem cells present in human PDL and gingiva. The findings of the current study indicated that both GMSCs and PDLSCs have similar basic cellular characteristics (morphology, CFUs, PDT, and cell surface markers) and mesenchymal differentiation (adipogenic and osteogenic) potential. Each of the parameters that were studied is discussed in detail in the following paragraphs.

In this study, isolation was performed using the combination of enzymatic degradation and explant methods as previously reported by Iriate *et al.*, which demonstrated that this method showed superior

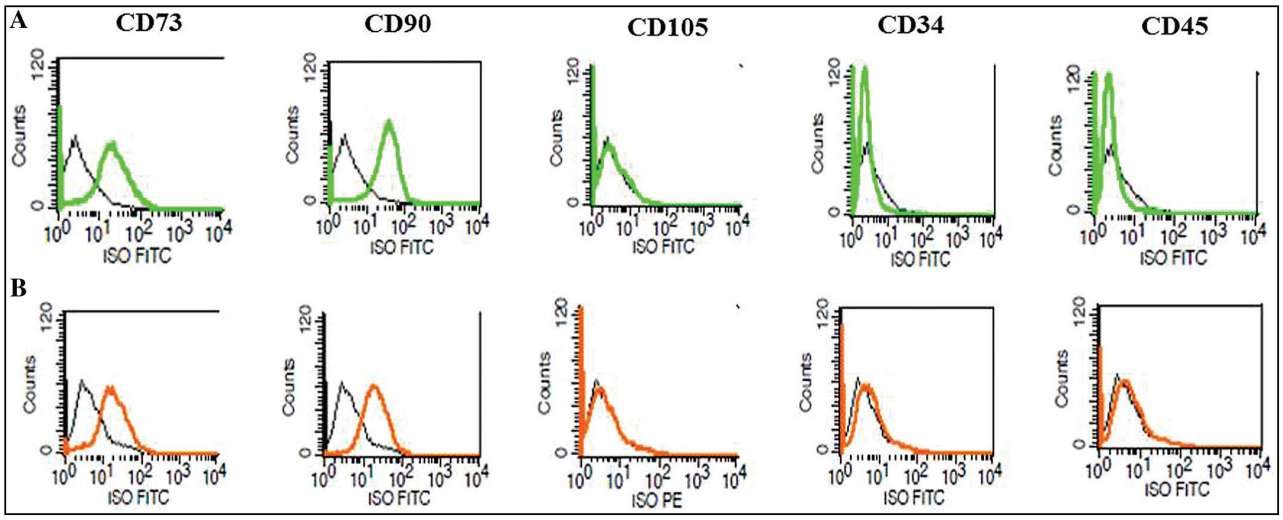

Figure 5: Flow cytometry analysis of stem cell marker expression in GMSCs (A) and PDLSCs (B). Cells were stained with antibody against CD73, CD90, CD105, CD34, and CD45. In merged images, dark-lined histograms indicate signal of isotype control, and green and orange-lined histograms indicate the positive reactivity with stained specific antibody. A total of 10,000 cells were analyzed for each sample in duplicates. Representative examples indicating marker expression profiles are presented

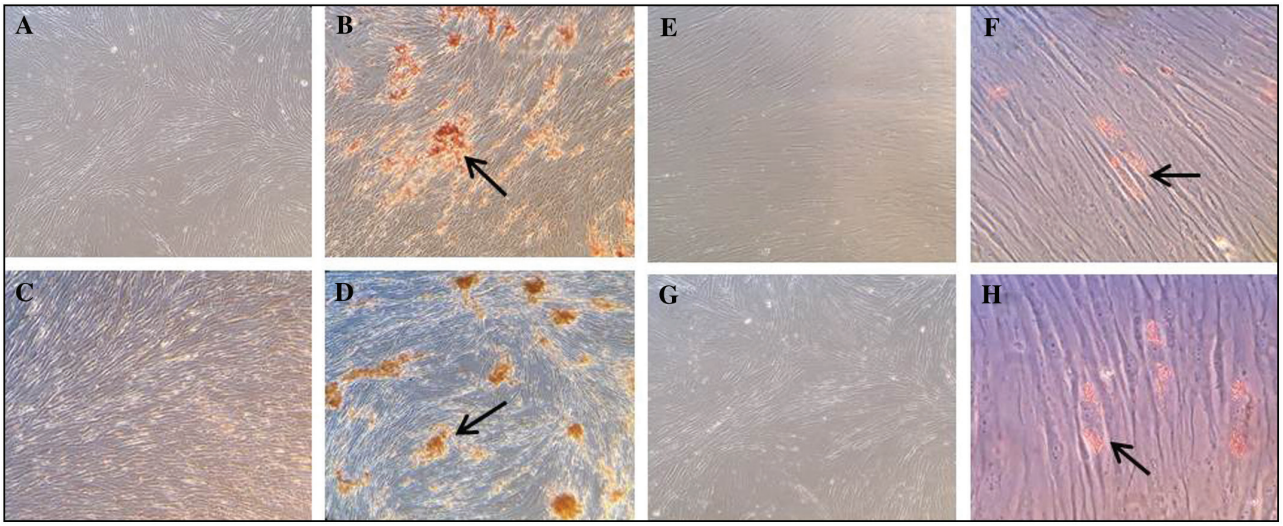

Figure 6: Osteogenic and adipogenic differentiation potential of GMSCs and PDLSCs. (A and C) GMSCs and PDLSCs without osteogenic induction medium (control). (B and D) Images indicating the calcium deposition and mineralization of nodules by osteocytes (arrows) differentiated from GMSCs and PDLSCs after 3 weeks induction and demonstrated by Alizarin red-S staining (arrows) (×20). (E and G) Cells with no adipogenic induction medium. (F and H) Formation of intracellular lipid droplets in adipocytes was observed when GMSCs and PDLSCS were cultured in adipogenic induction media for 3 weeks. Fat globules presence was confirmed by oil red-O staining (arrows) (×20)

population harvest.[18] Other studies have used the enzyme degradation method and explant methods to obtain cell lines for periodontal regeneration with adequate success. The successful primary culture was established with both gingival connective tissue and PDL samples in this study.[16,19,20] Both GMSC and PDLSC exhibited fibroblast-like morphology and after two weeks reached confluence after which they were trypsinized for subculture.

The CFU is a measure of viable clonogenic cell numbers. CFU indicates the number of cells that remain viable enough to proliferate and form small colonies in culture. In this study, colony formation could be seen in GMSC [Figure 2B], whereas it was not apparent in PDLSC. This showed that GMSC seemed to exhibit greater replicative potential. This was in accordance with a study by Seo *et al*.,[6] where CFU was low for PDLSCs. Also, Yang *et al.* in their study found that CFU was higher for GMSCs than PDLSCs to an extent where the PDLSCs count was too low for further experimentation.[21] However, some earlier studies have shown that PDLSC has a high frequency of CFU as attributed to its fibrous nature.[7,22] Furthermore, cell viability is used to monitor the response and health of cells in culture. In this study, the results showed that >98% of GMSCs and >97% of PDLSCs in all the passages were viable and making both the cells highly capable of growth and function.

PDT is one of the most important parameters of clinical-grade cell culture. This is a precise way to measure cell growth kinetics. When plated at low densities, GMSCs and PDLSCs exhibited their ability to proliferate at higher rates. The average PDT for PDLSCs was found to be slightly lower than GMSCs. The average PDT with respect to GMSCs shown in this study was similar to the observations made by Tomar *et al.* and Yang *et al.* found a slight reduction in the PDT, and increased proliferation and differentiation capacity were observed in PDLSCs compared with GMSCs.[21,23]

The results of cell surface markers analysis showed that GMSCs and PDLSCs were highly positive for CD73, CD90 and weakly positive for CD105. Earlier studies have shown that CD105 (endoglin) expression varies with source and a more negative expression is suggestive of a tendency to differentiate into adipocytes and osteocytes.[24] Vasandhan *et al.* showed that there was a low expression of CD105 in PDLSCs similar to this study and they postulated that this may be due to more intrinsic quality of the cell type than as a result of differences in culture.[25] Literature has evidenced that PDLSCs and GMSCs are usually strongly positive for CD73, CD90, and CD105, considered as MSC-specific markers.[7,10-12,18] The expression of CD34 and CD45 was almost negative in this study for both GMSC and PDLSC. These were employed as the negative markers to exclude the presence of hematopoietic stem cells and the results are similar to many other studies.[6,11,12,15,21] However, CD34 expression appears to depend on its environment and can change from positive to negative, and vice versa, as they move between tissue compartments.

The mesenchymal lineage differentiation potential of GMSCs and PDLSCs was evaluated in this study. GMSCs when cultured in osteogenic and adipogenic induction media for three weeks, showed the formation of calcium deposition and lipid vacuoles, respectively. This was similar to previous studies where GMSCs showed their ability to differentiate into osteocytes and adipocytes.[12,16] A recent study by Sun *et al.* established GMSCs as a promising source for bone tissue engineering. Conversely, a study by Yang *et al.* demonstrated that the capability of the GMSCs to undergo osteogenic differentiation was slightly decreased compared with PDLSCs.[21] In addition, PDLSCs when cultured in osteogenic and adipogenic induction media showed the formation of calcium deposition through mineralized nodules and neutral lipids, respectively. This was similar to other studies in which PDLSCs differentiated in response to adipogenic and osteogenic stimuli.[6,7,22] But the findings were not in accordance with a study conducted by Vasandhan *et al.* who showed that PDLSCs were completely unresponsive to adipogenic stimuli and did not form mineralized nodules and calcium deposits in response to osteogenic stimuli. They postulated that this could be because they might require additional signaling from pathways like Notch to establish terminal differentiation to osteoblasts or because of the absence of CD105.[25]

Therefore, in this study, it was clearly demonstrated that GMSCs represent MSC population and have a similar proliferative potential to PDLSCs. A comparative study carried out by Santamaria *et al.* also showed that GMSCs prove to have similar proliferative potential as PDLSCs.[26] However, unlike our study, they used different individuals as sources of PDL and gingiva, cultured the tissue using the enzymatic degradation method, and checked for *in vivo* tumorigenicity. Abedian *et al.* also showed a similar study comparing GMSCs and PDLSC of third molars using MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay but suggested that PDLSCs maybe better for therapeutic approaches in tissue engineering.[27]

Another recent study done on rats confirmed that GMSC-CM (conditioned medium) transplantation could significantly promote periodontal regeneration and achieves the same effect as PDLSC-CM.[28]

In vitro and *in vivo* evidence has shown that the PDLSCs are involved in the regulation of homeostasis of the periodontium and the promotion of its regeneration. It has been shown that these PDLSCs are capable of multilineage differentiation into fibroblasts, osteoblasts, cementoblasts, adipocytes, and so forth.[7,8] Human clinical trials have also started which in selected cases show that PDLSCs may be safe and efficacious in promoting regeneration of diseased defects and tissues after periodontitis.[10,11] However, harvesting of PDLSCs has drawbacks such as low yield and the need of extracting the tooth. Thus, other sources, including gingiva have been researched as alternate cell sources.[11,12,16]

Gingival tissue could be accessed more easily as a byproduct of routine dental surgery without necessitating the extraction of a tooth. Zhang *et al.* first isolated a population of progenitor cells within gingival tissue that formed clonogenic colonies expressed a typical MSC surface marker profile and possessed the ability to differentiate into multiple lineages *in vitro.* Thus, this identification of GMSCs represented a more widely available cell population with immense potential for therapeutic applications.[10,15]

Certain limitations of the study maybe

1. Sample size could be increased.
2. Present study was a comparison of basic cellular and biological characteristics only. However, molecular analysis on certain marker expressions could help to strengthen the study.
3. The differentiation potential was measured by qualitative approach only whereas an additional quantitative assessment could be made.

In conclusion, this study established a primary culture of GMSCs and PDLSCs from the same patient to avoid donor variations in separate samples and found that GMSCs could be a viable alternative to PDLSCs in cell-based therapy.

Acknowledgement

We would like to acknowledge the team at Nitte University Centre for Stem cell research and regenerative medicine (NUCSReM) who have supported us with the necessary equipments and manpower.

Financial support and sponsorship

The work was partially supported by Nitte (Deemed to be University) - NUFR2 grant of Rs. 1,00,000/-

Conflicts of interest

There are no conflicts of interest.

Authors contributions

Not applicable.

Ethical policy and institutional review board statement

The study was approved by the Ethics Committee of AB Shetty Memorial Institute of Dental Sciences (Cert No. ABSM/EC87/2015), Nitte (Deemed to be University), and the Nitte University Institutional Committee for Stem Cell Research (IC-SCR No.NU/ICSCR/2016–17/002A/P1).

Patient declaration of consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Data availability statement

Not applicable.

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