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Comparative characterization and analysis of telomere length in stem cells derived from deciduous and permanent teeth

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ABSTRACT

Background: Understanding the influence of age on growth kinetics and telomere length in dental stem cells is essential for the successful development of cell therapies. Hence, the present study compared the basic cellular and phenotypical characteristics of stem cells from human exfoliated deciduous teeth (SHEDs) and dental pulp stem cells (DPSCs) of permanent teeth and their telomere lengths using quantitative real-time polymerase chain reaction.

Materials and Methods: The study is an *in vitro* original research article. Primary cultures of SHED and DPSCs (n = 6 each) were successfully established *in vitro*, and the parameters analyzed were the morphology, viability, proliferation rate, population doubling time (PDT), phenotypic markers expression, and the relative telomere lengths. Data were analyzed by analysis of variance and $P < 0.05$ was considered statistically significant.

Results: SHED and DPSCs exhibited a small spindle-shaped fibroblast-like morphology with >90% viability. The proliferation assay showed that the cells had a typical growth pattern. The PDT values of SHED and DPSCs were 29.03 ± 9.71 h and 32.05 ± 9.76 h, respectively. Both cells were positive for surface markers CD29, CD44, and CD90. However, they were negative for CD45 and human leukocyte antigen DR. Although the differences in relative telomere lengths between the individual cell lines of SHED and DPSCs were observed, no significant ($P > 0.05$) variations were found for the mean T/S ratios of both the cells.

Conclusion: SHED and DPSCs displayed similar morphology, proliferation rates, and phenotypic features. The relative telomere lengths were slightly shorter in DPSCs than SHED, but the values were not significantly different. Thus, SHED and DPSCs can be considered as recognized sources for regenerative applications in dentistry.

Key Words: Deciduous teeth, dental pulp, stem cells, telomere

INTRODUCTION

In recent years, multipotent mesenchymal stem/stromal stem cells (MSCs) are the most clinically relevant population in the adult stem cell category with their potential use in cellular therapy to replace damaged cells or to regenerate organs.[1] In this direction, the isolation and characterization of a distinctive type of MSCs have been made from

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different types of human dental tissues, such as dental pulp stem cells (DPSCs),[2-4] stem cells from human exfoliated deciduous teeth (SHED),[5-7] periodontal ligament stem cells,[8] gingiva derived mesenchymal stem cells (GMSCs),[9] alveolar bone-derived MSCs,[10] stem cells from apical papilla (SCAP),[11] and dental follicular stem cells.[12,13] These cells possess MSC characteristics including the capacity for self-renewal, expression of the cell surface markers and alkaline phosphatase activity, and ability for multilineage differentiation potential.

Among the dental stem cells (DSCs), SHED and DPSCs have shown to possess the properties of high proliferative potential, self-renewal, and multilineage differentiation.[14] However, for the application of stem cells in tissue engineering and regenerative medicine, it is important to optimize their isolation and preserve their phenotypic properties *in vitro*.[14,15] Moreover, it is essential to determine the influence of donor age on stem cells for the successful development of cell therapies as it has been demonstrated that the cell numbers decline with donor age.[16-18] In addition to decrease in overall expansion potential, earlier investigations have also documented that stem cells from aged have a decreased proliferation rate and telomere length compared to young from the initial cell passages.[16,18,19] Furthermore, the telomere shortening has been shown to inhibit the mobilization of stem cells out of their niche.[20] Collectively, these studies highlighted the role of telomere and telomerase as critically important biological features of normal tissue stem/progenitor cells.

Telomeres are specialized structures in the ends of linear chromosomes capped with a series of tandem repeat noncoding TTAGGG sequences that are protected within a higher order nucleoprotein complex.[17,21] These telomeres protect chromosome from end-to-end fusions, recombination, and degeneration.[21] However, with each cell division, the most distal telomere repeats are lost as a result of end-replication problem.[18] It has been well recognized that the maintenance of telomere length in pluripotent and multipotent stem cells is a necessary factor for their self-renewal and lineage differentiation.[17,18,21,22] In this regard, long-term proliferative ability of DSCs is limited and during their *in vitro* expansion, these cells tend to show the slowdown in proliferation and might progress to replicative senescence.[13,16,17] However, for the application of DSCs in cell-based therapies, high cell number is necessary. To obtain stem cells in large numbers, it is imperative to preserve the self-renewal capacity and differentiation ability under *in vitro* culture conditions. Keeping the above research requirements in view, the present study investigated the basic cellular and phenotypical characteristics of SHED and DPSCs and then compared their telomere lengths using quantitative real-time polymerase chain reaction (real-time PCR).

MATERIALS AND METHODS

The present study is an *in vitro* research article that compared the basic cellular and phenotypical characteristics of SHED and DPSCs along with their relative telomere lengths.

Materials

All chemicals were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA) and media from Gibco (Invitrogen, Life Technologies, Thermo Fisher Scientific, Grand Island, NY, USA), unless otherwise specified.

Ethics statement

The present study followed the Declaration of Helsinki on medical protocol and ethics and was approved by the Institutional Ethics Committee and the Institutional Committee for Stem Cell Research.

Sample collection

Healthy exfoliated deciduous teeth up to two-thirds of root resorption were obtained from six children with an age from 5 to 12 years and free from disease or previously treated endodontically. Further, healthy, noncarious first premolar teeth were collected from six adults aged 18–24 years. Informed written consents were obtained before the procedures were performed.

Isolation and culture of stem cells from human exfoliated deciduous teeth and dental pulp stem cells

Freshly extracted teeth were washed thrice with Dulbecco's phosphate-buffered saline solution (DPBS), and the pulp tissues were gently separated from the pulp chamber and root canal and isolated using a sterilized broach. The pulp was then rinsed in DPBS with 100 U/mL of penicillin, 100 µg/mL of streptomycin, and 2.5 µg/mL of amphotericin B, mined into fragments of 0.5–1 mm^3 and then digested in a mixture of 0.1% collagenase I for 30 min at 37°C. Following centrifugation at 1200 rpm for 5 min, the cell pellet was obtained along with partially digested pulp tissue remnants. These explants were placed on

plastic tissue culture dishes containing a complete medium (Modified Eagle's medium-alpha [MEM-α]) supplemented with 10% fetal bovine serum (FBS), 100 U/mL of penicillin, 100 μg/mL of streptomycin, and 2.5 μg/mL of amphotericin B and cultured at 37°C in a humidified atmosphere of 5% CO_2 in air. Once the pulp tissue explants were firmly adhered to the surface of the culture dish, fresh MEM-α with 10% FBS, 100 U/mL of penicillin, and 100 μg/mL of streptomycin were changed once in 3 to 4 days of culture. The sliced remnants of pulp tissues were removed after confirming the outgrowth of plastic adherent cells during 48–96 h. When the cells reached 90% confluence approximately after 2 weeks in incubation, sub-passaging by 0.25% trypsin-ethylenediaminetetraacetic acid was performed and cultured up to fifth passages for performing the analyses.

Morphology and viability assay

Cell morphology of SHED and DPSCs was assessed at different passages using a phase-contrast microscope (Olympus, Japan). Cells were gently washed with DPBS to remove floating population in the culture before photomicrography. The cell viability was assessed in triplicates using 0.4% trypan blue. The stained cells were counted in a hemocytometer with phase-contrast microscope (Olympus).

Analysis of proliferation and population doubling time

To analyze the proliferation and population doubling time (PDT) of SHED and DPSCs, culture-expanded cells were seeded in a range from 1×10^4 cells/well in a 12-well culture plate (Thermo Fisher Scientific, USA). The cells from each well were harvested on days 3, 6, 9, and 12 and counted with hemocytometer under phase-contrast microscope (Olympus). Three sets of cultures at defined passage were performed and the mean counts were calculated. The PDT at that particular passage was determined using a formula PDT = t (log 2)/(log Nt-log No), where t represents culture time, No and Nt represent the cell number before and after seeding, respectively.

Flow cytometry analysis of cell surface markers

SHED and DPSCs were harvested at third passage and 3×10^5 cells were incubated with each antibody, including CD29 (eBioscience, 1:100), CD44 (Biolegend, CA, USA, 1:100), CD90 (eBioscience, CA, USA, 1:100), CD45 (eBioscience, CA, USA, 1:100), and human leukocyte antigen (HLA)-DR (Biolegend, CA, USA, 1:100) for flow cytometry analysis (BD FACSCalibur, Becton Dickinson, USA). Following washes with cell staining buffer, fluorescein isothiocyanate-conjugated anti-mouse IgG used as a secondary antibody was stained for 1 h at room temperature. Nonlabeled cells and isotypes were used as negative controls. A total of 10,000 events were collected and analyzed with Cell Quest software (Becton Dickinson).

Isolation of DNA from stem cells from human exfoliated deciduous teeth and dental pulp stem cells

Genomic DNA (gDNA) was extracted by phenol: chloroform: isoamyl alcohol (PCI) method. Briefly, one volume of PCI (25:24:1 v/v) was added to the cell suspension containing 1×10^6 cells and vortexed vigorously for 30 s. Then, it was centrifuged at room temperature for 5 min at 16,000 g and the aqueous layer obtained was carefully transferred into a new 1.5 mL centrifuge tube. After which DNA precipitation was achieved by adding 100% ethanol and overnight incubation at −20°C. The following day, the sample was centrifuged at 4°C for 30 min at 16,000 g and the supernatant was discarded without disturbing the gDNA pellet. Then, the pellet was washed with 70% ethanol and centrifuged at 4°C for 2 min at 16,000 g. The remaining ethanol was removed as much as possible and the DNA pellet was air-dried at room temperature for 5–10 min. Finally, the gDNA was resuspended in 50 μL of nuclease-free water by pipetting up and down several times and was quantified using a nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, USA).

Quantification of relative telomere length by real-time polymerase chain reaction

Telomere length analysis (TLA) was performed by real-time PCR (Applied Biosystems, StepOnePlusTM) using SYBR® Premix Ex Taq TM II (Takara, Japan) following previously published protocols with minor modifications.[23,24] Briefly, each reaction, per DNA sample, was carried out in triplicates. The reaction mixture of 10 μL was consisted of 5 μL master mix, 0.2 μL forward primer, 0.2 μL reverse primer, 100 ng gDNA template, and nuclease-free water. The cycling conditions were 10 min of initial denaturation at 95°C, 35 cycles of 94°C for 30 s, 60°C for 1 min followed by a dissociation stage. Beta-globin was used as a single copy gene (SCG), and the primer sequences used for the amplifications are presented in Table 1. gDNA from the MCF7 cell line was used as a reference sample. From the values obtained, mean Ct for each telomere (TEL) and SCG for every sample was calculated and the

relative T/S ratio was expressed as $2^{-\Delta\Delta Ct}$. ΔΔCT method was used to evaluate the relative fold expression of target genes, which was normalized against the expression level of beta-globin for every sample.

Statistical analysis

All data were presented as the mean ± standard deviation (SD) from at least three independent experiments and one-way or two analysis of variance was performed by GraphPad Prism software (GraphPad, CA, USA). Significance was considered at $P < 0.05$.

RESULTS

Establishment of primary cultures and morphology

Primary cultures of SHED and DPSCs were successfully established *in vitro* from the collected deciduous and premolar teeth samples, respectively. The representative images of SHED and DPSCs are presented in Figure 1. Both SHED and DPSCs firmly attached onto the plastic culture dishes and exhibited a small spindle-shaped fibroblast-like morphology during the primary culture (passage 0). However, compared to SHED, DPSCs demonstrated a slightly broader and elongated morphology in the later passages (passage 5). Both cells reached confluency by 15–18 days in primary culture and also by 12 days in subsequent passages. Overall, under primary culture and during expansion, SHED and DPSCs displayed more or less similar morphological features.

Table 1: Primers used for telomere length analysis by real-time polymerase chain reaction

Primer name	Primer sequence (5'-3')
HBB	F-GCTTCTGACACAACTGTGTTCACTAGC
	R-CACCAACTTCATCCACGTTCACC
IFNB1	F-GGTTACCTCCGAAACTGAAGA
	R-CCTTTCATATGCAGTACATTAGCC
Human TELO	F-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTTT
	R-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT
Human TELOGC	F-ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT
	R-TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA

HBB: Human beta-globin, IFNB1: Human interferon beta 1, TELO: Telomere, TELOGC: TelomericGC

Viability

The viability of SHED and DPSCs was assessed at different time points of passage 3 by trypan blue exclusion assay and the values are graphically presented in Figure 2a. The results showed >90% viability in both the cells, and statistical analysis showed no significant ($P > 0.05$) differences between SHED and DPSCs.

Proliferation rate and population doubling time

The proliferative potential of SHED and DPSCs was analyzed by deriving a growth curve [Figure 2b] and PDT [Figure 2c]. Both the cells displayed a typical growth curve consisting of initial lag phase (0–4 days), steep exponential phase (5–9 days) and finally reached the plateau in the end (10–12 days). PDT of SHED was 29.03 ± 9.71 h and that of DPSCs were 32.05 ± 9.76 h. No statistically significant difference ($P > 0.05$) was observed in the proliferation rate and PDT values of SHED and DPSCs.

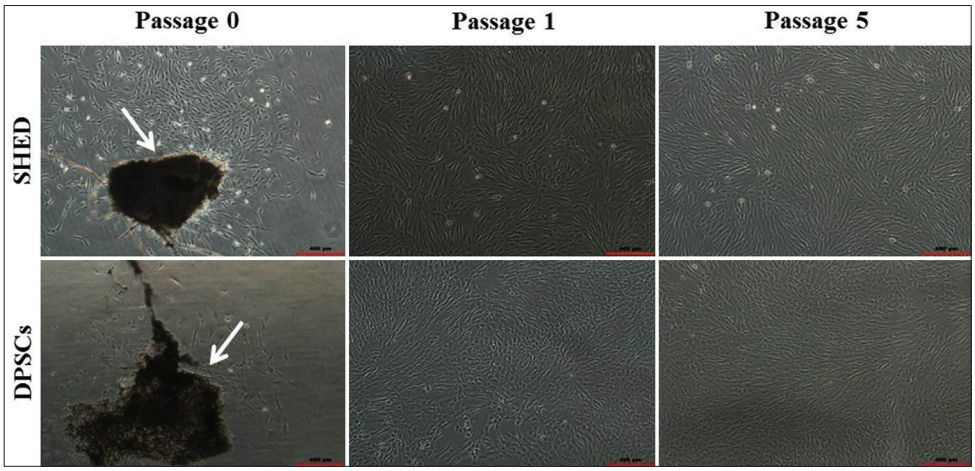

Figure 1: Morphology of stem cells from human exfoliated deciduous teeth and dental pulp stem cells. Both stem cells from human exfoliated deciduous teeth and dental pulp stem cells exhibited small spindle-shaped fibroblast-like morphology from their onset of release in the primary culture (Passage 0, arrows). However, unlike stem cells from human exfoliated deciduous teeth, dental pulp stem cells displayed a slightly broader morphology in the later passages (P5).

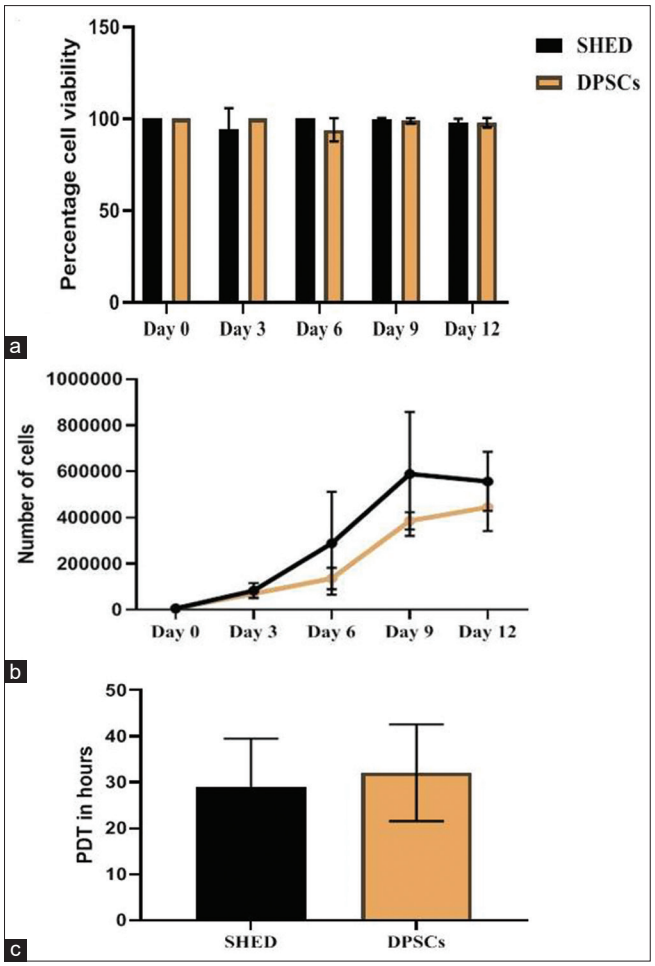

Figure 2: Viability, proliferation rate, and population doubling time of stem cells from human exfoliated deciduous teeth and dental pulp stem cells. (a) >90% viability was observed in both the cells. (b) stem cells from human exfoliated deciduous teeth and dental pulp stem cells displayed typical growth curves. (c) population doubling time of stem cells from human exfoliated deciduous teeth and dental pulp stem cells presented in hours. Values are presented in mean ± standard deviation. No statistically significant difference ($P > 0.05$) was observed.

Expression of cell surface markers

The representative images of the expression of cell surface markers in SHED and DPSCs by flow cytometry analysis are depicted in Figure 3. Results clearly exhibited that SHEDs were positive for CD29 (95.09%), CD44 (99.03%), and CD90 (74.40%) and negative for CD45 (0.40%) and HLA-DR (0.85%). Further, the data showed that DPSCs were also positive for CD29 (97.44%), CD44 (97.88%), and CD90 (75.06%) and negative for CD45 (0.20%) and HLA-DR (1.32%).

Telomere length analysis of stem cells from human exfoliated deciduous teeth and dental pulp stem cells

TLA was performed using real-time PCR method. From the Ct values of TelomericGC (TELOGC) and SCG (beta-globin), T/S ratio was calculated for each sample. MCF7 gDNA sample was used as telomere control for normalization. The TLA values in SHEDs varied from 0.6898 to 1.2583 [Figure 4a]. The SHEDs analyzed for relative telomere length had a significant ($P < 0.05$) difference among the individual cell lines and also compared to telomere control. The TLA values in DPSCs varied from 0.6914 to 1.1961 [Figure 4b]. The relative telomere length of DPSCs showed a significant ($P < 0.05$) difference among the individual cell lines, except between DPSCs-1 and -2, and DPSCs-4 and -5. The values of DPSCs were also significantly different ($P < 0.05$) when compared to telomere control.

Comparison of telomere length analysis between stem cells from human exfoliated deciduous teeth and dental pulp stem cells

After analyzing the TLA of SHED and DPSCs ($n = 6$ each), the comparison was made and the levels are presented as mean ± SD using MCF7 as a reference [Figure 4c]. The mean T/S ratios for SHED and DPSCs were 1.0061 and 0.9551, respectively, and no significant ($P > 0.05$) difference was observed in these levels. However, the levels of SHED and DPSCs were significantly ($P < 0.05$) higher when compared to the reference cell line, MCF7 (0.7524).

In addition, the efficiency of telomere amplification by TELO primers (TELO and TELOGC) and SCGs (human beta-globin and human interferon-beta 1) was confirmed by gel electrophoresis [Figure 5]. The amplification of genes in real-time PCR products was reconfirmed by detecting the bands at specific base pairs in MCF7, SHED, and DPSCs.

DISCUSSION

The present study compared the cellular and biological properties of SHED and DPSCs along with telomere lengths. Both cells were successfully established under *in vitro* conditions and employed for the assessment of morphology, viability, proliferation rate and PDT, and the expression of phenotypic markers, such as CD29, CD44, CD90, CD45, and HLA-DR. Further, they were subjected for the assessment of telomere length by real-time PCR.

In this study, both SHED and DPSCs exhibited a small spindle-shaped fibroblast-like morphology from their onset of release in the primary culture. However, unlike SHED, DPSCs displayed a slightly broader and elongated morphological features in

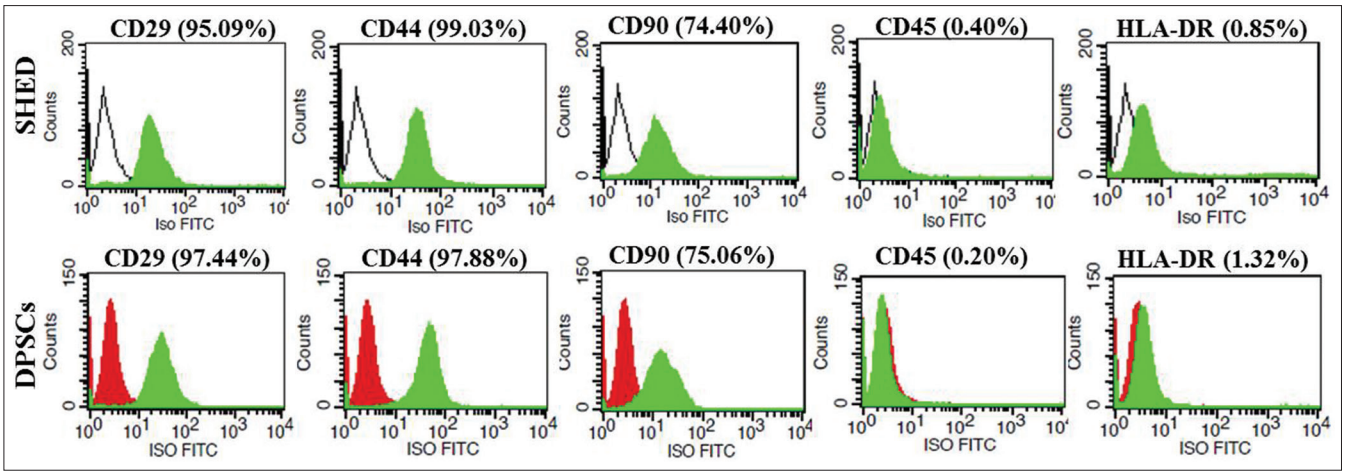

Figure 3: Expression of cell surface markers in stem cells from human exfoliated deciduous teeth and dental pulp stem cells by flow cytometry analysis. The representative plots are showing the staining proportion of selected markers and isotype. The cell surface markers are represented as filled-green histograms and isotype control is represented as dark-lined or filled-red histograms. Expression level of each antibody is presented.

the later passages (Passage 5). Apart from this, no noticeable differences were observed during their culture expansion. In addition, SHED and DPSCs showed >90% viability with no significant ($P > 0.05$) differences at different time points of assessment. The observations of morphological features and live cells are consistent with that of previous results, indicating the MSC-like features of SHED and DPSCs.[2,3,5-7,14]

The growth kinetics of SHED and DPSCs in terms of proliferation rate and PDT presented a typical growth pattern. The PDT values of SHED and DPSCs were 29.03 ± 9.71 h and 32.05 ± 9.76 h, respectively, and no differences were observed in their proliferation rates. Earlier reports indicated that SHED possesses more clonogenic and proliferative potentials than DPSCs.[5,6] SHEDs have been shown to be distinct from DPSCs due to their higher proliferation rate and increased cell population doubling rates.[6,14,15] In contrast, the growth characteristics of the cells obtained from both the permanent and deciduous teeth sources were similar.[25] However, there was a difference in the ratio of fibroblastoid cells to epithelioid cells between the cultures obtained from both the sources. Our results suggest that SHED and DPSCs are comparable on their cellular properties and may have originated from similar precursors. Therefore, the proliferative ability of both cells can be considered as an important feature for their usage in clinical protocols.

Flow cytometry analysis was performed for determining the expression of cell surface markers in SHED and DPSCs. The data clearly showed that both cells were positive for MSC-specific surface antigens CD29, CD44, and CD90. However, these cells were negative for hematopoietic markers, CD45, and HLA-DR. Importantly, the cells were strongly positive for a marker of myoepithelial differentiation, CD29 (neural lineage development of neural stem cell, neural crest, and neuronal populations from human stem cells), early adhesion and hyaluronan marker, CD44, and cell–cell and cell–matrix interactions, CD90. With these expression patterns, the isolated SHED and DPSCs displayed the minimal criteria for defining MSCs as proposed by the International Society for Cellular Therapy.[26] In addition, the expression profile is consistent with that of previous results, indicating MSC-like phenotypic features of SHED and DPSCs.[5-7,14,17] Given that the SHED and DPSCs were derived from dental pulp tissues of marginally different age groups, the phenotypic markers expression results were comparable and did not exhibit any marked differences.

The present study investigated the telomere lengths in SHED and DPSCs as it has been recognized that cell proliferation ability is closely linked to the telomere status.[13,16-18] The real-time PCR-based TLA provided the relative data of telomere length by calculating the ratio of a PCR reaction from the same sample (SHED or DPSCs) using specific primers for telomeres and SCG (T/S ratio).[23,24] The relative telomere length values in SHEDs and DPSCs varied from 0.6898–1.2583 to 0.6914–1.1961, respectively. The values largely showed significant ($P < 0.05$) differences among the individual cell lines, except between

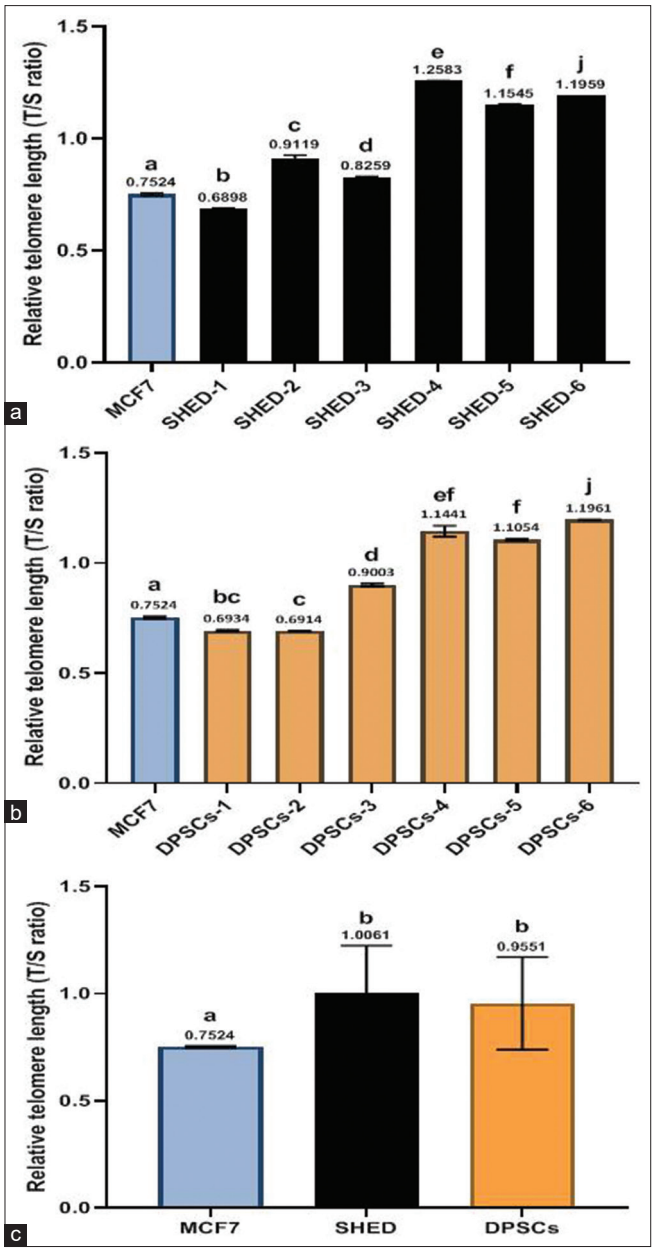

Figure 4: Telomere length analysis of stem cells from human exfoliated deciduous teeth and dental pulp stem cells. The relative telomere length of stem cells from human exfoliated deciduous teeth (a) and dental pulp stem cells (b) showed a significant difference ($P < 0.05$) among the different cell lines and also compared to MCF7. However, the combined average relative telomere length of stem cells from human exfoliated deciduous teeth and dental pulp stem cells showed no significant differences ($P > 0.05$). (c) Values are presented in mean ± standard deviation. Different superscripts indicate statistically significant difference at $P < 0.05$.

DPSCs-1 and -2 and DPSCs-4 and -5. However, the mean T/S ratios for SHED and DPSCs were 1.0061 and 0.9551, respectively, and no significant ($P > 0.05$) difference was observed. Further, the levels of SHED and DPSCs were significantly ($P < 0.05$) higher when compared to the reference cell line, MCF7 (0.7524).

In this study, it was found that cellular and phenotypic characteristics of stem cells obtained from different pulp tissue sources, such as deciduous and permanent teeth, were relatively similar, but differences might exist in terms of telomere length when individual cells lines were considered. The minor differences in the values of telomere length could be explained by the varied status of isolated cells under culture expansion period. As the regenerative potential of isolated SHED and DPSCs depends on their proliferative ability and capacity to undergo differentiation, the stem cell populations might exhibit a minor heterogeneity during *in vitro* culture expansion because of the differences in sample origin, developmental stage, culture techniques in the laboratory and media composition and also dissimilarities in the donor age.[13,17-19,27]

Earlier findings demonstrated that *ex vivo* expansion of adult stem cells should be minimal to avoid detrimental effects on telomere maintenance, and measurement of telomere length should become a standard when certifying the status and replicative age of stem cells before therapeutic applications.[28] However, it was observed that proliferative and regenerative heterogeneity are related to contrasting telomere lengths and CD271 expression between DPSC populations.[4] The results suggested that these characteristics may ultimately be used to selectively screen and isolate high proliferative capacity/multipotent DPSCs for regenerative medicine. Further, it has been suggested that short telomere length and increased DNA damage with genomic instability correlate with the accelerated induction of cellular senescence.[13] In this study, similarities were observed in terms of the proliferative ability and telomere length of SHED and DPSCs. Relative telomere length was slightly shorter in DPSCs than SHED, as expected, but the values were not significantly ($P > 0.05$) different.

CONCLUSION

In the present study, SHED and DPSCs displayed a similar morphology, proliferation rate, and phenotypic characteristics. Although the differences in relative telomere lengths (T/S ratio) between the individual cell lines of SHED and DPSCs were observed, no variations were found for the mean T/S ratios. These similarities were in accordance with the proliferative

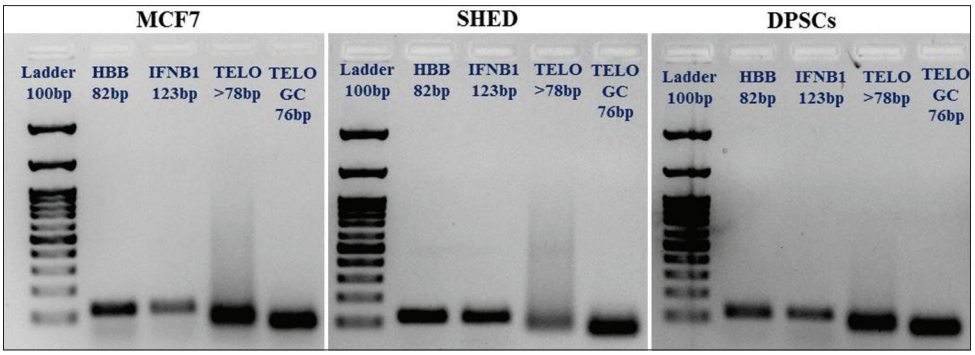

Figure 5: Efficiency of telomere amplification by TELO primers (TELO and TELOGC) and single copy genes (human beta-globin and human interferon-beta 1). Images of agarose gel electrophoresis of real-time polymerase chain reaction products reconfirmed the amplification by exhibiting the bands at specific base pairs in MCF7, stem cells from human exfoliated deciduous teeth, and dental pulp stem cells.

abilities of SHED and DPSCs under *in vitro* culture conditions. Thus, the results suggested that SHED and DPSCs can be considered as recognized sources for regeneration and repair in dentistry. However, individual differences in SHED and DPSCs should be identified and taken into consideration before their applications in clinical therapy.

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Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

REFERENCES

1. Gomez-Salazar M, Gonzalez-Galofre ZN, Casamitjana J, Crisan M, James AW, Péault B. Five decades later, are mesenchymal stem cells still relevant? Front Bioeng Biotechnol 2020;8:148.
2. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human Dental Pulp Stem Cells (DPSCs) *in vitro* and *in vivo*. Proc Natl Acad Sci U S A 2000;97:13625-30.
3. Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, Boyde A, *et al.* Stem cell properties of human dental pulp stem cells. J Dent Res 2002;81:531-5.
4. Alraies A, Alaidaroos NY, Waddington RJ, Moseley R, Sloan AJ. Variation in human dental pulp stem cell ageing profiles reflect contrasting proliferative and regenerative capabilities. BMC Cell Biol 2017;18:12.
5. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, *et al.* SHED: Stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A 2003;100:5807-12.
6. Wang X, Sha XJ, Li GH, Yang FS, Ji K, Wen LY, *et al.* Comparative characterization of stem cells from human exfoliated deciduous teeth and dental pulp stem cells. Arch Oral Biol 2012;57:1231-40.
7. Chhabra R, Rao S, Kumar BM, Shetty AV, Hegde AM, Bhandary M. Characterization of stem cells from human exfoliated deciduous anterior teeth with varying levels of root resorption. J Clin Pediatr Dent 2021;45:104-11.
8. Gay IC, Chen S, MacDougall M. Isolation and characterization of multipotent human periodontal ligament stem cells. Orthod Craniofac Res 2007;10:149-60.
9. Zhang Q, Shi S, Liu Y, Uyanne J, Shi Y, Shi S, *et al.* Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. J Immunol 2009;183:7787-98.
10. Matsubara T, Suardita K, Ishii M, Sugiyama M, Igarashi A, Oda R, *et al.* Alveolar bone marrow as a cell source for regenerative medicine: Differences between alveolar and iliac bone marrow stromal cells. J Bone Miner Res 2005;20:399-409.
11. Zhang W, Zhang X, Ling J, Liu W, Zhang X, Ma J, *et al.* Proliferation and odontogenic differentiation of BMP2 gene-transfected stem cells from human tooth apical papilla: An *in vitro* study. Int J Mol Med 2014;34:1004-12.
12. Morsczeck C, Götz W, Schierholz J, Zeilhofer F, Kühn U, Möhl C, *et al.* Isolation of Precursor Cells (PCs) from human dental follicle of wisdom teeth. Matrix Biol 2005;24:155-65.
13. Morsczeck C, Reck A, Reichert TE. Short telomeres correlate with a strong induction of cellular senescence in human dental follicle cells. BMC Mol Cell Biol 2019;20:5.
14. Shi X, Mao J, Liu Y. Pulp stem cells derived from human permanent and deciduous teeth: Biological characteristics and therapeutic applications. Stem Cells Transl Med 2020;9:445-64.
15. Rosaian AS, Rao GN, Mohan SP, Vijayarajan M, Prabhakaran RC, Sherwood A. Regenerative capacity of dental pulp stem cells: A systematic review. J Pharm Bioallied Sci 2020;12:S27-36.
16. Ng TK, Chen CB, Xu C, Xu Y, Yao X, Huang L, *et al.* Attenuated regenerative properties in human periodontal ligament-derived stem cells of older donor ages with shorter telomere length and lower SSEA4 expression. Cell Tissue Res 2020;381:71-81.
17. Jeon BG, Kang EJ, Kumar BM, Maeng GH, Ock SA, Kwack DO, *et al.* Comparative analysis of telomere length, telomerase and reverse transcriptase activity in human dental stem cells. Cell Transplant 2011;20:1693-705.
18. Fathi E, Charoudeh HN, Sanaat Z, Farahzadi R. Telomere shortening as a hallmark of stem cell senescence. Stem Cell

Investig 2019;6:7.
19. Yu JM, Wu X, Gimble JM, Guan X, Freitas MA, Bunnell BA. Age-related changes in mesenchymal stem cells derived from rhesus macaque bone marrow. Aging Cell 2011;10:66-79.
20. Marędziak M, Marycz K, Tomaszewski KA, Kornicka K, Henry BM. The influence of aging on the regenerative potential of human adipose derived mesenchymal stem cells. Stem Cells Int 2016;2016:2152435.
21. Hiyama E, Hiyama K. Telomere and telomerase in stem cells. Br J Cancer 2007;96:1020-4.
22. Serakinci N, Graakjaer J, Kolvraa S. Telomere stability and telomerase in mesenchymal stem cells. Biochimie 2008;90:33-40.
23. Cawthon RM. Telomere measurement by quantitative PCR. Nucleic Acids Res 2002;30:e47.
24. Vasilishina A, Kropotov A, Spivak I, Bernadotte A. Relative human telomere length quantification by real-time PCR. Methods Mol Biol 2019;1896:39-44.
25. Shekar R, Ranganathan K. Phenotypic and growth characterization of human mesenchymal stem cells cultured from permanent and deciduous teeth. Indian J Dent Res 2012;23:838-9.
26. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, *et al.* Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315-7.
27. Trivanović D, Jauković A, Popović B, Krstić J, Mojsilović S, Okić-Djordjević I, *et al.* Mesenchymal stem cells of different origin: Comparative evaluation of proliferative capacity, telomere length and pluripotency marker expression. Life Sci 2015;141:61-73.
28. Mokry J, Soukup T, Micuda S, Karbanova J, Visek B, Brcakova E, *et al.* Telomere attrition occurs during ex vivo expansion of human dental pulp stem cells. J Biomed Biotechnol 2010;2010:673513.