Talebi *et al.*
European Journal of Medical Research (2025) 30:407
https://doi.org/10.1186/s40001-025-02670-9

European Journal
of Medical Research

RESEARCH

Open Access

Growth and size of stem cells from human exfoliated deciduous teeth cultured in platelet lysate vs. fetal bovine serum for regenerative applications

Ardeshir Talebi[1], Sanaz Razi[1], Sahar Talebi[2*], Nosrat Nourbakhsh[3*], Hossein Talebi[4], Sareh Soroushzadeh[5], Samin Razi[2] and Seyed Ali Mosaddad[6,7,8]

Abstract

Background Stem cells from human exfoliated deciduous teeth (SHED) can differentiate into functioning neurons and oligodendrocytes and exhibit immunomodulatory and regenerative properties. This study aimed to compare the growth and size of SHED cultured in human platelet lysate (hPL) and fetal bovine serum (FBS), with the goal of evaluating their potential use in regenerative medicine.

Methods Healthy deciduous teeth with uninflamed pulp were used for culture. Growth, proliferation, and morphology of the cultured SHED, as well as the mean surface area, length, and width of the cells, were compared between FBS and hPL.

Results The mean surface area of the cultured SHED was lower on FBS than on hPL on days 4, 6, and 9 of passage 0, higher on FBS on day 2, and comparable on days 5 and 10. Comparison of growth, proliferation, and morphology of the cultured SHED on FBS and hPL on days 3 and 9 of passage 0 showed no significant difference. In addition, there was no significant difference regarding growth, proliferation, and morphology of the cells between FBS and hPL on the same days of passages 1, 2, 3, and 4. The total mean length of the cells was lower on hPL than on FBS within 10 days (137.9 vs. 148.1 µm), while the total mean width of the cells was higher on hPL than on FBS within 10 days (28 vs. 26 µm).

Conclusions FBS and hPL were comparable regarding growth, proliferation, and morphology of SHED, with higher mean width and lower mean length on hPL than on FBS within 10 days and overall comparable mean surface area after 10 days of passage 0. Therefore, FBS and hPL appear to be relatively similar for the culture of SHED, and hPL can replace FBS for this purpose.

Keywords Fetal bovine serum, Human platelet lysate, SHED

*Correspondence:
Sahar Talebi
sahar.talebi.dds@gmail.com
Nosrat Nourbakhsh
Full list of author information is available at the end of the article

Background

Stem cells from human exfoliated deciduous teeth (SHED) are highly self-renewing cells from the cranial neural crest that reside in the dental pulp's perivascular niche [1]. According to prior research, SHED may differentiate into functioning neurons and oligodendrocytes under the right circumstances and exhibit immunomodulatory and regenerative properties [2, 3]. These cell types are readily accessible via noninvasive techniques, can be efficiently differentiated into osteoblasts, odontoblasts, adipocytes, chondrocytes, and neural cells both in vitro and in vivo, and display minimal immunological responses or rejection after SHED transplantation. In addition, SHED may continue to be undifferentiated and stable even after prolonged cryopreservation [4].

Fetal bovine serum (FBS) is commonly employed as a supplement in the isolation and expansion of stem cells due to its widespread use in cell culture protocols and its role in supporting cell growth in research and preclinical studies, including those that may involve cells intended for clinical applications [5, 6]. FBS is a rich source of nutrients, hormones, and growth factors; however, it also has a high endotoxin concentration and may be contaminated with microorganisms, including viruses, bacteria, fungi, and prions [7]. Furthermore, it has been shown that individuals who have received cell transplants using mesenchymal stromal cells (MSCs) grown on FBS have antibodies against bovine antigens [8]. In addition, there are ethical considerations regarding FBS acquisition [9]. Thus, many factors derived from humans have been proposed as substitutes for FBS to provide growth factors and nutrients for cell culture [10].

As an alternative to animal serum, human platelet lysate (hPL) has been suggested [11, 12]. Growth factors such as transforming growth factor (TGF), epidermal growth factor (EGF), and platelet-derived growth factor (PDGF) are present in hPL [13], as well as lipids, glucose, electrolytes, albumin, fibrinogen, and other coagulation factors [14]. If hPL is produced from platelet concentrates obtained only from approved manufacturers, these ingredients may impact the cultivated cells and end up in the cell product [15]. The ex vivo propagation of multiple MSCs has been performed for research and medical applications [16, 17].

Moreover, for tissue regeneration to occur, it is crucial to choose the appropriate scaffold to supply sites of tissue cell adhesion, sustain cell proliferation and differentiation, and ultimately promote tissue regeneration. Materials used as scaffolds are typically three-dimensional in shape and are intended to closely resemble natural extracellular matrices in every aspect [18].

Given the drawbacks of FBS, the development of hPL as a replacement for FBS, and the significance of SHED culture, this study aimed to compare the growth and morphology of SHED cultured in hPL and FBS media to assess their potential for future regenerative applications.

Methods

Study design

This was an in vitro study on children's samples, performed on Stem Cells from Human Exfoliated Deciduous Teeth (SHED) cultured in fetal bovine serum (FBS) (Sigma Aldrich) and human platelet lysin (HPL) (BioScience, Aachen) cell culture media. The inclusion criteria for deciduous teeth were children aged between 6 and 9 years, teeth with physiologic mobility near exfoliation, and incisor deciduous teeth without any carious, pulpal treatment or trauma history. The teeth were extracted under local anesthesia in all subjects, and a pediatric dentist extracted eligible teeth. It is worth noting that the parents willingly provided their children's teeth for research purposes and completed the informed consent forms from Isfahan University of Medical Sciences. The study received approval from the Ethics Committee of Isfahan University of Medical Sciences (IR.MUI.REC.1400.042) and complies with the statements of the Declaration of Helsinki.

Stem cell isolation and culture

Forty extracted teeth samples were placed in 50 ml Falcon tubes containing 20 ml phosphate buffer saline (PBS) (Gibco, UK) and Penicillin/Streptomycin 2% (Gibco, USA). Then, the samples were transferred to the central laboratory of Isfahan University of Medical Sciences. There, the Falcon tubes were thoroughly disinfected with alcohol. The dental pulp was isolated using barbed broaches size #2 (Rojin, China) from apical root resorption. The pulp samples were then moved to 3400 ml evaporating dishes containing PBS. The dental pulp was cut into small pieces using a scalpel and transferred to a centrifuge tube containing an enzyme solution composed of 3 mg/mL collagenase (Sigma Aldrich) and 3 mg/mL dispase (Sigma Aldrich) dissolved in 1 mL of PBS. The tissue was subjected to enzymatic digestion, and the resulting cell suspension was filtered to remove undigested fragments. Next, the tube containing the enzyme solution and dissected pulp tissue was placed in a water bath at 37 °C for 1 h. To purify the cells obtained from the enzymatic process, a nylon mesh filter with pores of 40–70 µm was placed over two different centrifuge tubes containing 3 mL PBS. The solution containing relatively digested samples was gently poured onto the nylon mesh, allowing it to pass through slowly. In the next step, the filtered solution in the centrifuge tubes was centrifuged at 1700 RPM for 5 min. After centrifugation, the supernatant was removed from the two tubes, and the remaining

pellet at the bottom of each tube was slowly broken with finger strokes. Then, by adding the desired media (FBS or hPL) to each tube, AlphaMEM (Sigma Aldrich) supplemented with FBS serum at a 20% concentration, 1% glutamine (Gibco, UK), 1% non-essential amino acid (Gibco, UK), and 1% antibiotic pen/strep solution for the FBS media group, and AlphaMEM supplemented with hPL serum at a 10% concentration, 1% glutamine, 1% non-essential amino acid, and 1% antibiotic pen/strep solution for the hPL media group. The cell suspension was cultured in the specified cells of a 12-well plate and placed in an incubator at 37 °C, 95% humidity, and 5% CO_2.

Passage

When the bottom of the dish was covered by cells, and the desired 80–90% cell confluency was achieved, cell passaging was performed. Cells were washed twice with 1 mL PBS. Then, 1 mL of 0.05% trypsin–EDTA enzyme (Gibco, UK) was added to each dish for 4 min to detach cells from the culture substrate. The culture medium containing 10% FBS/hPL was added to the dish to neutralize the enzyme's activity. The cell suspension was transferred to a centrifuge tube and centrifuged at 1700 RPM for 8 min. The supernatant fluid was removed from the tubes, and the remaining pellet at the bottom of each tube was slowly broken with a finger tab. One of the two desired culture media was added to the broken cell sediments and transferred to several new dishes according to the cell density. The new dishes were placed into an incubator at 37 °C with 95% humidity and 5% carbon dioxide. If necessary, the remaining cells were frozen with media containing 90% FBS/hPL and 10% DMSO (Sigma Aldrich) in liquid nitrogen at −196 °C for later use or long-term storage.

Measurements

1. Cell count: After staining with trypan blue, the unstained cells were counted using NeoBar slides.
2. Phenotype: To evaluate the phenotype, antibodies against surface markers CD90, CD73, and CD105 were used, and flow cytometry was performed, whose results have been previously published [19].
3. Growth: Growth was determined by the number of days it took for the culture media to fill up.
4. Viability: The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to evaluate viability. For this purpose, at a density of 5000 cells/cm^2, SHEDs were placed in a 96-well plate. Then, they were cultivated using 200 µl of PL or FBS. Viability was assessed on the seventh day using 1 mg/ml MTT solution. This solution was added to each well, and the plate was kept in the incubator for 2 h. Then, the solution was removed and replaced by 1 ml of isopropanol in each well. Subsequently, the plate was shaken for 30 min at room temperature to dissolve formazan crystals. Next, 200 µl isopropanol was poured into a 96-well plate as a control. Finally, a spectrophotometer was used to evaluate absorption at 570 nm. Prior to conducting the present study, a pilot experiment was performed to evaluate the viability of SHED cultured in both FBS and hPL media. Viability was calculated using the following formula: (test optical density (OD)/control OD) × 100. After 7 days of culture, the mean viability of SHED cultured in FBS was 91.7 ± 3.2%, while those cultured in hPL demonstrated a mean viability of 93.1 ± 3.6%. No statistically significant differences were observed between the groups ($P > 0.05$), confirming that both FBS and hPL maintain high viability rates under identical culture conditions. Based on these preliminary findings, the current study focused on comparing SHED proliferation and growth dynamics in these two culture environments, using confluence as the criterion for growth assessment. All in vitro experiments, including cell counting, growth, viability, and morphological assessments, were performed in triplicate and repeated independently three times ($n = 3$) to ensure reproducibility and statistical validity of the results.

Cell morphology measurements

The length, width, and surface area of cultured SHED were measured using ImageJ software (National Institutes of Health, USA). For each experimental group, 100 cells were randomly selected from microscopic images captured under identical conditions. The major (length) and minor (width) diameters of each cell were measured in microns. To estimate the surface area, each cell was considered as a rhombus, and the area was calculated using the formula: (major diameter × minor diameter)/2. This calculated area approximates the actual surface area of the cell when adherent to the culture dish.

All in vitro experiments, including cell counting, growth, viability, and morphological assessments, were performed in triplicate and repeated independently three times ($n = 3$) to ensure reproducibility and statistical validity of the results.

Data analysis

The SPSS software (version 25.0) was used for data analysis. The independent t test was used to compare continuous variables between groups. P values < 0.05 were regarded as significant in all statistical tests.

Results

The mean surface area of the cultured SHED was lower on FBS than on hPL on days 4, 6, and 9 of passage 0, higher on FBS on day 2, and comparable on days 5 and 10 (Fig. 1). Figure 2 shows the surface area of the cultured SHED on FBS and hPL on day 10 of passage 0. Comparison of growth, proliferation, and morphology of the cultured SHED on FBS and hPL on days 3 and 9 of passage 0 showed no significant difference (Fig. 3). In addition, there was no significant difference regarding growth,

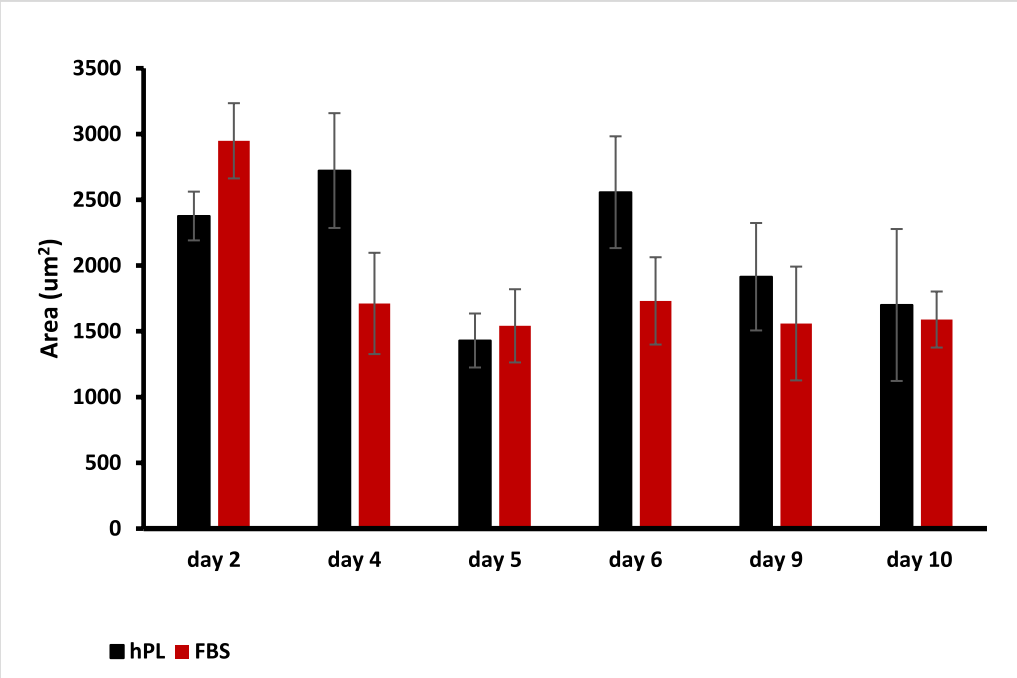

Fig. 1 Mean surface area of the cultured SHED on FBS and hPL on days 2, 4, 5, 6, 9, and 10 of passage 0 (error bar indicates standard error of the mean)

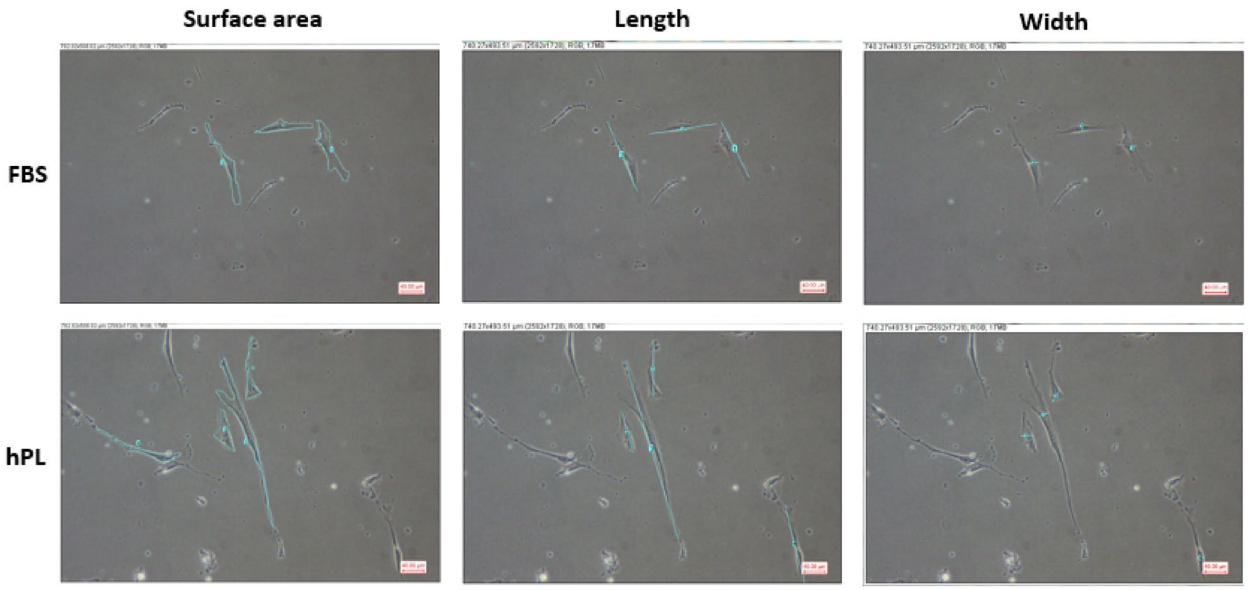

Fig. 2 Surface area, length, and width of the cultured SHED on FBS and hPL on day 10 of passage 0

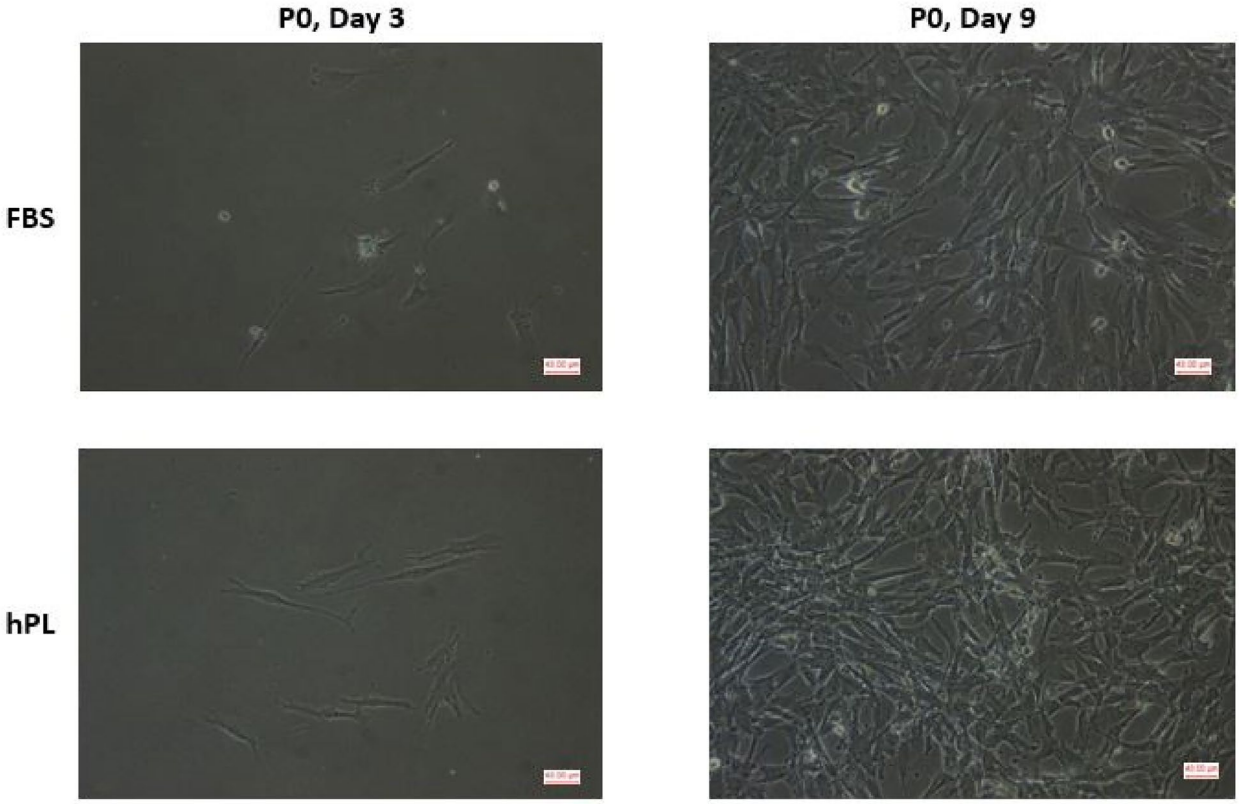

Fig. 3 Growth, proliferation, and morphology of the cultured SHED on FBS and hPL on days 3 and 9 of passage 0

proliferation, and morphology of the cells between FBS and hPL on the same days of passages 1, 2, 3, and 4 (Fig. 4).

The morphological assessment of SHED cultured in hPL and FBS media revealed differences in cell dimensions over the 10-day period. The mean length of SHED cultured in hPL was lower than that of cells cultured in FBS (137.9 µm vs. 148.1 µm), while the mean width was slightly higher in hPL compared to FBS (28 µm vs. 26 µm) (Table 1; Fig. 2).

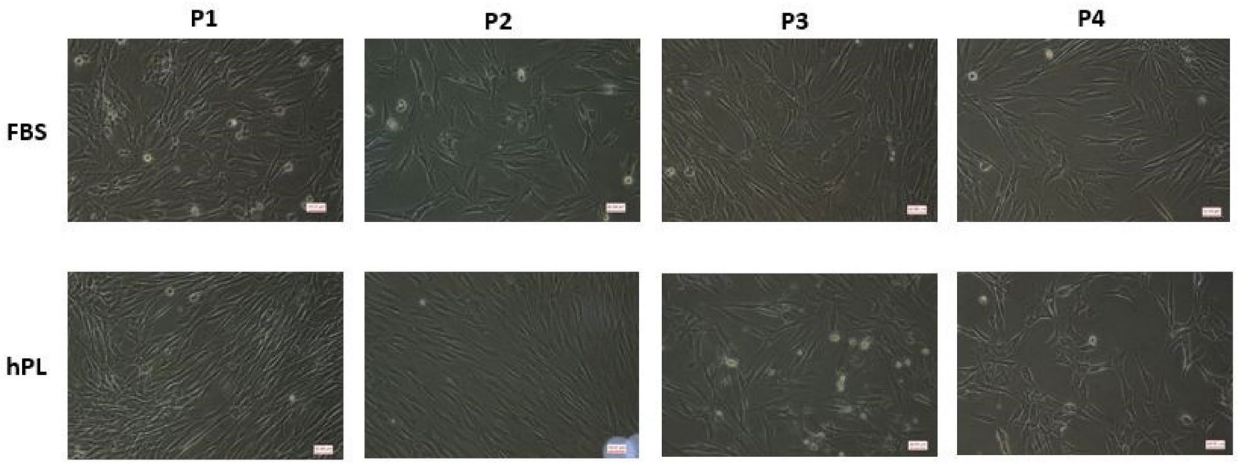

Fig. 4 Growth, proliferation, and morphology of the cultured SHED on FBS and hPL on the same days of passages 1, 2, 3, and 4

Table 1 Mean length and width of the cultured SHED on FBS and hPL

Days	Mean length (μm)			Mean width (μm)		
	hPL	FBS	Mean	hPL	FBS	Mean
2	138.7	94.3	116.5	34.4	28.5	31.4
4	158.0	157.5	157.8	39.4	24.8	32.1
5	152.2	140.0	146.1	21.0	27.0	24.0
6	110.0	179.0	144.5	28.8	21.7	25.2
9	134.8	138.8	136.8	27.6	32.4	30.0
10	133.6	179.0	156.3	16.8	21.7	19.2
Mean	137.9	148.1	142.9	28.0	26.0	26.9

FBS fetal bovine serum, *hPL* human platelet lysate, *SHED* stem cells from human exfoliated deciduous teeth

Discussion

An essential objective of stem-cell research is to identify high-quality human postnatal stem cells from readily available resources. Several tissues, including skeletal muscle, bone marrow, brain, skin, hair follicles, and dental pulp, have been used for the isolation of postnatal stem cells [20–22]. SHED is a source of postnatal stem cells that are highly self-renewing and can differentiate into a variety of cell types, including osteogenic, odontogenic, adipogenic, and neural cells [23]. On the other hand, the role of adult stem cells is to replace lost tissue by producing new cells. Adult stem cells must be able to preserve the tissue stem cell pool and develop into tissue-specific somatic cells to fulfill this regenerative ability throughout life [24]. The asymmetric cell division feature of the stem cells enables them to divide and leave their daughter cells in an undifferentiated condition to replenish the stem cell pool, which is called "self-renewal" [24]. As mentioned above, self-renewal is a property of SHED. Recent studies have further highlighted the regenerative potential of dental stem cells, including SHED, for clinical applications. Stem cells derived from dental tissues have been explored for their ability to differentiate into various cell types and support tissue regeneration when combined with appropriate biomaterials and scaffolds. In this regard, SHED has been recognized as a promising candidate for the regeneration of bone, dental, and neural tissues due to its accessibility and high proliferative capacity [25]. Moreover, the role of signaling pathways, such as Wnt/β-catenin, in enhancing the regenerative functions of dental stem cells has gained increasing attention. A recent study [26] demonstrated that activation of the Wnt pathway via Tideglusib significantly influenced the expression of type I and III collagen in dental pulp-derived stem cells, suggesting a modulatory role in extracellular matrix remodeling, which is essential for tissue engineering and regeneration [22]. These findings underscore the importance of optimizing culture conditions and understanding signaling mechanisms to fully harness the regenerative capabilities of SHED for future clinical applications.

The current study showed relatively similar results for FBS and hPL regarding the culture of SHED. FBS and hPL were comparable regarding growth, proliferation, and morphology of SHED, with higher mean width and lower mean length on hPL than on FBS within 10 days and overall comparable mean surface area after 10 days of passage 0. FBS and hPL have been compared for the culture of MSCs in previous studies. In harmony with this study, by comparing hPL with FBS for culture and endothelial differentiation of human amniotic fluid MSCs (hAF–MSCs), Tancharoen et al. showed that comparable to those grown in 10% FBS-supplemented medium, hAF–MSCs exhibited similar growth characteristics, MSC marker expression, and specific protein localization when grown in 10% hPL-supplemented media [27]. Similarly, Favaloro et al. reported that apheresis hPL may work as a good substitute for FBS because of the analogous development and phenotype of bone marrow MSC grown in ether medium (commercial hPL or apheresis hPL) [28]. In another study, Mohammadi et al. used hPL as a xeno-free alternative to FBS for the in vitro expansion of human MSCs and demonstrated that hPL could serve as a suitable and safe substitute for FBS in the development of MSCs without causing any clonal chromosomal abnormalities in the expanded cells [12]. It is also important to consider that enzymatic passaging may exert stress on stem cells and contribute to alterations in morphology or function [29]. Although a low concentration of trypsin and a short exposure time were used to minimize cellular damage, the process of repeated passaging can lead to telomere shortening and reduced stemness [30]. This highlights the importance of limiting passage number in experimental protocols and considering alternative, gentler detachment methods in future studies.

MSCs are often cultured in FBS, although this technique raises questions regarding the possibility of xenogeneic protein transfer from the animal-derived serum [11]. Human platelet lysate has been suggested as an alternative to FBS [11, 12]. When cutting-edge culture systems for human stem cells are used, such as when high FBS content in culture media is replaced by non-animal derived components like hPL, the stem cell properties of self-renewal and multipotency must be maintained under those culture conditions [31]. The current study showed that these properties were preserved when hPL was used for the culture of SHED. Ranuch et al. also showed a comparable growth-promoting effect of hPL with FBS for adipose-derived MSCs while maintaining their undifferentiated state [32], which confirmed their findings from an earlier study [7].

This study presents several limitations that should be considered when interpreting the results. The sample size was limited to healthy deciduous teeth from children aged 6–9 years, which may not represent other age groups or individuals with varying systemic or dental health conditions, thus restricting the generalizability of the findings. The evaluation period for growth and morphology was limited to 10 days, which may not reflect the long-term viability, differentiation capacity, and stability of SHED cultured in human platelet lysate and fetal bovine serum. It is also important to note that the SHED used in this study was derived from children aged 6–9 years, during the physiological exfoliation phase. At this stage, the stem cell population may exhibit enhanced biological activity, including increased transdifferentiation potential and responsiveness to environmental cues, due to their involvement in the natural process of root resorption (rizolysis) [1, 33, 34]. This inherent property may influence the generalizability of the results to SHED derived from other developmental stages. Although the study was conducted under controlled in vitro conditions, these may not replicate the complexity of in vivo environments, where immune responses, vascularization, and mechanical forces influence stem cell behavior. Moreover, only two types of culture media were compared, and other possible supplements or combinations that might enhance SHED proliferation and differentiation were not explored. Although standardized manual counting methods were used in this study, the incorporation of automated cell counting systems could further improve measurement accuracy and reduce operator-dependent variability. Finally, natural variability in the source of deciduous teeth, including differences in donor health status and tooth type, could influence the outcomes and affect reproducibility across different studies.

Future research should address these limitations through broader sampling, longer observation periods, in vivo validation, the evaluation of alternative culture conditions, and the use of automated platforms to enhance reproducibility in cell quantification and reduce subjective bias. Future studies should also consider the inclusion of a serum-free culture condition to serve as a negative control. This would provide valuable information regarding the intrinsic proliferative capacity of SHED and allow for a clearer assessment of the specific contributions of serum-derived growth factors in supporting cell expansion and morphology. In addition, incorporating such a control would establish a baseline for evaluating changes in cell morphology, helping to isolate the effects of FBS and hPL on cellular structure and behavior. Furthermore, the inclusion of flow cytometric analysis to assess the expression of mesenchymal stem cell surface markers (e.g., CD73, CD90, and CD105) would allow for a more comprehensive characterization of the SHED population. Such analysis would provide further confirmation of stemness and lineage commitment, and is recommended as part of future studies to complement morphological and growth-based assessments.

Conclusions

FBS and hPL were comparable regarding growth, proliferation, and morphology of SHED, with higher mean width and lower mean length on hPL than on FBS within 10 days and overall comparable mean surface area after 10 days of passage 0. Therefore, FBS and hPL appear to be relatively similar for the culture of SHED, and hPL can replace FBS for this purpose. Since this was the first study to compare the two media for culturing SHED, extensive research is required to confirm these findings.

Data availability statement

The data presented in this study will be available on request from the corresponding author.

Acknowledgements
None.

Author contributions
Conceptualization: A.T and S.R; Methodology: A.T and S.R; Software: S.T and S.A.M; Validation: A.T, and N.N; Formal analysis: S.R, S.T, and H.T; Investigation: S.R, S.T, and H.T; Resources: A.T and N.N; Data Curation: S.S and S.R; Writing—Original Draft: S.R, S.S, and S.R; Writing—Review & Editing: A.T, N.N, and S.A.M; Visualization: S.R and S.R; Supervision: N.N; Project administration: A.T; Funding acquisition: A.T. All authors have read and approved the published version of the manuscript.

Funding
This study was funded by the Isfahan University of Medical Sciences, Isfahan, Iran.

Data availability
The data presented in this study will be available on request from the corresponding author.

Declarations

Conflicts of interest
The authors declare that they have no competing interests.

Ethical approval and consent to participate
The study originally titled "Comparison of Two Different Culture Media, PL and FBS, for Growth and Viability of SHED Stem Cells" was approved by the Research Ethics Committee of Isfahan University of Medical Sciences and Health Services on August 25, 2021 (approval number: IR.MUI.REC.1400.042). The study adheres to the principles outlined in the Declaration of Helsinki.

Consent for publication
Not Applicable.

Author details
[1]Department of Pathology, Medical School, Dental Research Center, Dental Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran. [2]School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran. [3]Department of Pediatric Dentistry, Dental Research Center, Dental Research Institute, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran. [4]Department of Cellular and Molecular Biology, Falavarjan Islamic Azad University, Isfahan, Iran. [5]Academic Center for Education, Culture and Research, Isfahan, Iran. [6]Department of Research Analytics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India. [7]Department of Prosthodontics, School of Dentistry, Shiraz University of Medical Sciences, Qomabad Street, Qasrodasht Avenue, Shiraz, Iran. [8]Department of Conservative Dentistry and Bucofacial Prostheses, Faculty of Odontology, University Complutense of Madrid, Madrid, Spain.

Received: 27 January 2025 Accepted: 8 May 2025
Published online: 21 May 2025

References

1. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S. SHED: Stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci USA. 2003;100(10):5807–12.
2. Sakai K, Yamamoto A, Matsubara K, Nakamura S, Naruse M, Yamagata M, et al. Human dental pulp-derived stem cells promote locomotor recovery after complete transection of the rat spinal cord by multiple neuro-regenerative mechanisms. J Clin Invest. 2012;122(1):80–90.
3. Arthur A, Rychkov G, Shi S, Koblar SA, Gronthos S. Adult human dental pulp stem cells differentiate toward functionally active neurons under appropriate environmental cues. Stem Cells. 2008;26(7):1787–95.
4. Saez DM, Sasaki RT, da Costa NA, da Silva MCP. Stem cells from human exfoliated deciduous teeth: a growing literature. Cells Tissues Organs. 2016;202(5–6):269–80.
5. Witzeneder K, Lindenmair A, Gabriel C, Höller K, Theiß D, Redl H, Hennerbichler S. Human-derived alternatives to fetal bovine serum in cell culture. Transfus Med Hemother. 2013;40(6):417–23.
6. Minonzio G, Linetsky E. The use of fetal bovine serum in cellular products for clinical applications: commentary. CellR4. 2014;2(6):e1307.
7. Rauch C, Feifel E, Amann E-M, Spötl HP, Schennach H, Pfaller W, Gstraunthaler G. Alternatives to the use of fetal bovine serum: human platelet lysates as a serum substitute in cell culture media. Altex. 2011;28(4):305–16.
8. Antoninus AA, Widowati W, Wijaya L, Agustina D, Puradisastra S, Sumitro SB, et al. Human platelet lysate enhances the proliferation of Wharton's jelly-derived mesenchymal stem cells. Biomark Genomic Med. 2015;7(3):87–97.
9. Jochems CEA, Van Der Valk JBF, Stafleu FR, Baumans V. The use of fetal bovine serum: ethical or scientific problem? Altern Lab Anim. 2002;30(2):219–27.
10. Bieback K, Hecker A, Kocaömer A, Lannert H, Schallmoser K, Strunk D, Klüter H. Human alternatives to fetal bovine serum for the expansion of mesenchymal stromal cells from bone marrow. Stem Cells. 2009;27(9):2331–41.
11. Doucet C, Ernou I, Zhang Y, Llense JR, Begot L, Holy X, Lataillade JJ. Platelet lysates promote mesenchymal stem cell expansion: a safety substitute for animal serum in cell-based therapy applications. J Cell Physiol. 2005;205(2):228–36.
12. Mohammadi S, Nikbakht M, Mohammadi AM, Panah MZ, Ostadali MR, Nasiri H, Ghavamzadeh A. Human platelet lysate as a xeno free alternative of fetal bovine serum for the in vitro expansion of human mesenchymal stromal cells. Int J Hematol Oncol Stem Cell Res. 2016;10(3):161.
13. Burnouf T, Strunk D, Koh MBC, Schallmoser K. Human platelet lysate: replacing fetal bovine serum as a gold standard for human cell propagation? Biomaterials. 2016;76:371–87.
14. Laner-Plamberger S, Oeller M, Mrazek C, Hartl A, Sonderegger A, Rohde E, et al. Upregulation of mitotic bookmarking factors during enhanced proliferation of human stromal cells in human platelet lysate. J Transl Med. 2019;17(1):1–13.
15. Schallmoser K, Henschler R, Gabriel C, Koh MBC, Burnouf T. Production and quality requirements of human platelet lysate: a position statement from the working party on cellular therapies of the international society of blood transfusion. Trends Biotechnol. 2020;38(1):13–23.
16. Oeller M, Laner-Plamberger S, Hochmann S, Ketterl N, Feichtner M, Brachtl G, et al. Selection of tissue factor-deficient cell transplants as a novel strategy for improving hemocompatibility of human bone marrow stromal cells. Theranostics. 2018;8(5):1421.
17. Uccelli A, Laroni A, Brundin L, Clanet M, Fernandez O, Nabavi SM, et al. MEsenchymal StEm cells for Multiple Sclerosis (MESEMS): a randomized, double blind, cross-over phase I/II clinical trial with autologous mesenchymal stem cells for the therapy of multiple sclerosis. Trials. 2019;20(1):1–13.
18. Zhang W, Walboomers XF, van Kuppevelt TH, Daamen WF, Bian Z, Jansen JA. The performance of human dental pulp stem cells on different three-dimensional scaffold materials. Biomaterials. 2006;27(33):5658–68.
19. Nourbakhsh N, Soleimani M, Taghipour Z, Karbalaie K, Mousavi SB, Talebi A, et al. Induced in vitro differentiation of neural-like cells from human exfoliated deciduous teeth-derived stem cells. Int J Dev Biol. 2011;55(2):189–95.
20. Bianco P, Kuznetsov SA, Riminucci M, Robey PG. Postnatal skeletal stem cells. Methods Enzymol. 2006;419:117–48.
21. Lee A, Kessler JD, Read T-A, Kaiser C, Corbeil D, Huttner WB, et al. Isolation of neural stem cells from the postnatal cerebellum. Nat Neurosci. 2005;8(6):723–9.
22. Kandyba E, Kobielak K. Wnt7b is an important intrinsic regulator of hair follicle stem cell homeostasis and hair follicle cycling. Stem Cells. 2014;32(4):886–901.
23. Yamaza T, Kentaro A, Chen C, Liu Y, Shi Y, Gronthos S, et al. Immunomodulatory properties of stem cells from human exfoliated deciduous teeth. Stem Cell Res Ther. 2010;1(1):1–11.
24. Snippert HJ, Clevers H. Tracking adult stem cells. EMBO Rep. 2011;12(2):113–22.
25. Mosaddad SA, Rasoolzade B, Namanloo RA, Azarpira N, Dortaj H. Stem cells and common biomaterials in dentistry: a review study. J Mater Sci Mater Med. 2022;33(7):55.
26. Güler C, Yilmaz AM, Kuru L, Ozen B, Agrali OB. The effect of tideglusib application on type 1 and type 3 collagen expressions by human dental-pulp derived stem cells: a preliminary study. Niger J Clin Pract. 2024;27(9):1065–72.
27. Tancharoen W, Aungsuchawan S, Pothacharoen P, Bumroongkit K, Puanінta C, Pangjaidee N, et al. Human platelet lysate as an alternative to fetal bovine serum for culture and endothelial differentiation of human amniotic fluid mesenchymal stem cells. Mol Med Rep. 2019;19(6):5123–32.
28. Favaloro J, Chen A, Kan F, Velickovic Z, Macpherson J, Loh Y, et al. Evaluation of human platelet lysate (HPL) as a substitute for foetal bovine serum (FBS) and recombinant proteins for the growth and maintenance of bone marrow derived mesenchymal stromal cells (BM-MSC). Cytotherapy. 2019;21(5):S84–5.
29. Badur MG, Zhang H, Metallo CM. Enzymatic passaging of human embryonic stem cells alters central carbon metabolism and glycan abundance. Biotechnol J. 2015;10(10):1600–11.
30. Wagner W, Horn P, Castoldi M, Diehlmann A, Bork S, Saffrich R, et al. Replicative senescence of mesenchymal stem cells: a continuous and organized process. PLoS ONE. 2008;3(5):e2213.

31. Bieback K. Platelet lysate as replacement for fetal bovine serum in mesenchymal stromal cell cultures. Transfus Med Hemother. 2013;40(5):326–35.
32. Rauch C, Wechselberger J, Feifel E, Gstraunthaler G. Human platelet lysates successfully replace fetal bovine serum in adipose-derived adult stem cell culture. J Adv Biotechnol Bioeng. 2014;2:1–11.
33. Sakai VT, Zhang Z, Dong Z, Neiva KG, Machado MA, Shi S, et al. SHED differentiate into functional odontoblasts and endothelium. J Dent Res. 2010;89(8):791–6.
34. Lee H-S, Jeon MJ, Kim S-O, Kim S-H, Lee J-H, Ahn S-J, et al. Characteristics of stem cells from human exfoliated deciduous teeth (SHED) from intact cryopreserved deciduous teeth. Cryobiology. 2015;71(3):374–83.

Publisher's Note