Journal of Advances in Biology & Biotechnology

25(3): 1-13, 2022; Article no.JABB.87039
ISSN: 2394-1081

Differential Viability in Alpha-MEM Culturing Media May Predict Alternative Media Responsiveness in Dental Pulp Stem Cell (DPSC)

Crystal Viss [aφ], Gavin Banning [b#], Sarah Swanbeck [b#] and Karl Kingsley [c*†]

[a] Department of Advanced Education, University of Nevada, Las Vegas - School of Dental Medicine, 1700 W. Charleston Blvd., Las Vegas, Nevada, 89106, United States of America.
[b] Department of Clinical Sciences, University of Nevada, Las Vegas - School of Dental Medicine, 1700 W. Charleston Blvd., Las Vegas, Nevada, 89106, United States of America.
[c] Department of Biomedical Sciences, University of Nevada, Las Vegas - School of Dental Medicine, 1001 Shadow Lane, Las Vegas, Nevada, 89106, United States of America.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2022/v25i330269

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/87039

Original Research Article

Received 07 March 2022
Accepted 09 May 2022
Published 13 May 2022

ABSTRACT

Objective: Dental pulp stem cells (DPSC) are the subjects of new and emerging fields of clinically applied biotechnology. However, much remains unknown regarding the most effective and appropriate methods for isolation, expansion and culture techniques for DPSC. To address these deficiencies, the primary objective of this study was to evaluate any effects of the major, commercially available cell culture media on DPSC phenotypes, such as growth, viability and biomarker expression.
Methods: This Institutional Review Board (IRB) approved study involved previously collected and cryopreserved DPSC isolates that were identified, thawed and cultured for this study (n=16). Each DPSC isolate was plated into 96-well assays under each of the experimental conditions (DMEM, DMEM:F12, RPMI, alpha-MEM) to determine any effects on cellular growth and viability. RNA was extracted from all DPSC isolates under the optimal growth conditions for screening using qPCR primers specific for DPSC biomarkers, such as Sox-2, Oct-4 and NANOG.

[φ]Pediatric Resident;
[#]Dental Student;
[†]PhD;
**Corresponding author: E-mail: karl.kingsley@unlv.edu;*

Results: Comparison of the standard DPSC cell culture media alpha-MEM to DMEM revealed differential results. Comparison of alpha-MEM to DMEM:F12 revealed no change among some DPSCs (n=3), decreased viability (n=8) or increased viability (n=5) - similar to the comparisons with RMPI demonstrating no change (n=5), decreased viability (n=6) or increased viability (n=5). Further analysis revealed that DPSC with low viability (<50%) in alpha-MEM responded positively to one or more of the culture media alternatives, while virtually none of DPSC with high viability (>50%) responded to any of the other experimental conditions. Screening of mRNA using qPCR revealed most DPSC isolates continued to express one or more of the pluripotent stem cell biomarkers (Oct4, Sox2, Nestin, NANOG), but no clear pattern of growth with the optimal media type correlated with viability.

Conclusions: These results demonstrated that many DPSC isolates responded positively to one or more of these media, including DMEM, DMEM:F12, RPMI when viability was <50% using the standard DPSC culture media alpha-MEM, but not when viability was >50%. These findings may be broadly applicable and add significantly to the evidence regarding the potential culturing methods that may be employed in various *ex vivo* and *in vitro* DPSC studies.

Keywords: Dental pulp stem cell; growth; viability; biomarkers.

1. INTRODUCTION

Dental pulp stem cells (DPSC) are pluripotent mesenchymal stem cells found in the interior of the "pulp chamber" within healthy intact teeth [1,2]. DPSCs may have many different and distinct functions within their microenvironment, such as the maintenance of pulp homeostasis and injury repair - including the formation of dentin by osteoblast-like progenitors [3,4]. Recent discoveries have demonstrated that these functions may be controlled, in part, by responses to complex sets of growth factors and scaffolds composed of extracellular matrix (ECM) proteins [5-7].

Evidence has emerged that DPSC may be capable of biological regeneration and tissue repair and are therefore the subject of intense research into these new and emerging fields of clinically applied oral and craniofacial biotechnology [8,9]. For example, much progress has been made in the field of periodontal ligament-derived mesenchymal stromal cells, which have been propagated *ex vivo* to regenerate and repair the periodontal attachment apparatus degradation induced by chronic disease and inflammation via bone grafting [10, 11]. Other research has demonstrated significant progress in DPSC use in neuronal and central nervous system (CNS) repair and regeneration, as well as retinal regeneration [12,13]. In fact, many studies now suggest that DPSCs may be useful in many types of biologic tissue repair and regeneration that are not restricted to the orofacial or craniofacial complex [14,15].

However, despite the progress made in recent years to demonstrate these potential applications, much remains unknown regarding the most effective and appropriate methods for isolation, expansion and culture techniques for DPSC [16,17]. In addition, it is not known whether viability and regenerative potential vary exclusively by DPSC phenotypes or biomarker expression or if it is these characteristics combined with isolation and culturing methods that determine therapeutic potential [18,19]. New studies are continuing to reveal new insights into culturing methods that modulate DPSC responsiveness and regeneration potential [20-22].

Studies from this group have evaluated aspects of DPSC viability and survival, including biomarkers and the effects of cryopreservation [23-26]. In addition, the various responses of DPSC to specific growth factors, such as vascular endothelial growth factor (VEGF), bone morphogenic protein (BMP) and transforming growth factor (TGF) have also been explored [27-30]. However, most researchers utilize a standard base media alpha-MEM when performing these studies [31-33]. Unfortunately, no systematic or side-by-side growth media studies have been conducted to determine if the commercially available growth media chosen has any significant effects on the viability, growth or other characteristics of DPSC.

To address these deficiencies, the primary objective of this study is to evaluate any effects of the major, commercially available cell culture medias (Dulbecco's Modified Eagle's Medium or DMEM, DMEM:F12, Roswell Park Memorial Institute or RPMI, and alpha-MEM) on DPSC phenotypes, such as growth, viability and expression of pluripotent stem cell biomarkers.

2. METHODS

2.1 Human Subjects

This study involved a retrospective analysis of previously collected dental pulp stem cell (DPSC) isolates from an existing biomedical repository, as previously described [31]. The protocol and procedures for this study were reviewed and subsequently approved by the Institutional Review Board (IRB) from the University of Nevada, Las Vegas (UNLV) under Protocol #171612-1 “Retrospective Analysis of Dental Pulp Stem Cells (DPSC) from the UNLV School of Dental Medicine (SDM) Pediatric and Clinical Population” on February 21, 2021.

2.2 Original Protocol

In brief, the original study protocol for the isolation of DPSC isolates was also reviewed and approved by the UNLV IRB and Office for the Protection of Research Subjects (OPRS) under Protocol-OPRS#0907-3148 “Isolation of Non-Embryonic Stem Cells from Dental Pulp" on February 5, 2010. Inclusion criteria included voluntary participation.

Briefly, patients scheduled for routine extractions of premolars or third molars (“wisdom teeth”) as part of their Orthodontic therapy were asked to participate. The extracted teeth were sectioned at the cementum-enamel junction (CEJ) and the exposed dental pulp was extracted using an endodontic broach for placement into sterile 1X phosphate buffered saline (PBS) for transfer to a biomedical laboratory. DPSC isolates were processed using the direct outgrowth method, as previously described [24-26]. Extracted RNA, which was isolated from each DPSC isolate, was screened for stem cell markers CD90 and CD105, as well as the absence of CD45 in accordance with the guidelines specified by the International Society for Cellular Therapy (ISCT), as previously described [24,25]. Expression of additional mesenchymal stem cell biomarkers (Sox-2, Oct-4 and NANOG) was also confirmed. Cells were passed for a minimum of ten (n=10) passages prior to cryopreservation in 10% dimethyl sulfoxide (DMSO)-containing media with Fetal Bovine Serum (FBS).

2.3 Cell Culture

For the current study, several previously collected and cryopreserved DPSC isolates were thawed and cultured for this study, n=18. In brief, each sample was thawed on ice, centrifuged at 2,100 x relative centrifugal force (RCF) for five minutes at room temperature and the DMSO-containing supernatant was removed prior to resuspension into each of the cell culture (experimental) conditions. In brief, each DPSC isolate was plated into 96-well assays under each of the experimental conditions, which included Dulbecco’s Modified Eagle’s Medium or DMEM, DMEM:Nutrient Mixture F12, Roswell Park Memorial Institute or RPMI, alpha-Minimum Essential Media or MEM (supplemented with 10% FBS and 1% Penicillin-Streptomycin) all from Gibco (Waltham, MA), to determine any effects on cellular growth and viability.

2.4 Proliferation and Viability Assays

All DPSC isolates were plated at 1.2 x 10^5 cells/mL in 96-well tissue culture treated flat bottom Corning Costar assay plates (Corning, NY) and allowed to proliferate in a biosafety level (BSL)-2 incubator at 37°C supplemented with 5% CO2. Cell viability was determined with the Trypan Blue exclusion assay using a BioRad TC20 automated cell counter. Absolute numbers and relative percentages of live cells were determined and exported into Microsoft Excel (Redmond, WA) for analysis.

Proliferation assays were fixed at 24 hours (one day), 48 hours (two days) or 72 hours (three days) with 10% formalin and processed using Gentian Violet 1% w/v alcoholic solution from RICCA Chemical Company (Arlington, TX). Assays plates were processed using a BioTek ELx808 microplate reader (Winooski, VT) at 630 nm and absorbance readings were exported into Microsoft Excel (Redmond, WA) for analysis.

2.5 RNA Extraction

Extraction of RNA from each DPSC isolate under each of the experimental conditions (DMEM, DMEM:F12, RPMI, and alpha-MEM was performed using the phenol:chloroform extraction method and the TriZol Reagent from ThermoFisher Scientific (Fair Lawn, NJ), as previously described [25,26,34]. RNA purity and quantification was measured using absorbance readings at A260nm and A280 nm with a NanoDrop spectrophotometer also from ThermoFisher Scientific. All samples were found to have A260:A280 ratios greater than or equal to 1.65, which is suitable for quantification using polymerase chain reaction (qPCR).

2.6 qPCR Screening

To determine if culturing media affects biomarker expression, RNA was screened using qPCR. In brief, samples were processed using the One-Step Reverse Transcription Kit from ThermoFisher Scientific. A Mastercycler gradient thermal cycler from Eppendorf (Hamburg, Germany) was used to facilitate reverse transcription for 30 minutes at 47°C. qPCR screening was then performed using reactions of 20 uL and the SYBR Green Master Mix from ThermoFisher Scientific. Each reaction contained 12.5 uL of ABsolute SYBR green, 7.5 uL of nuclease-free water, 1.75 uL of forward primer, 1.75 uL of reverse primer, and 1.5 uL of sample diluted to 1.0 ng/uL. Reactions included enzyme activation at 95°C for 15 minutes and 40 cycles consisting of denaturation at 95°C for 15 seconds, annealing at the primer pair-specific temperature for 30 seconds, and a final extension at 72°C for 30 seconds.

GAPDH control primers
GAPDH forward: 5′ATCTTCCAGGAGCGAGATCC-3′; 20 nt, 55% GC, Tm 66°C
GAPDH reverse: 5′ACCACTGACACGTTGGCAGT-3′; 20 nt, 55% GC, Tm 70°C
Optimal Tm: 61°C
Oct-4 forward: 5′-TGGAGAAGGAGAAGCTGGAGCAAAA-3′; 25 nt: 48% GC; Tm 70 °C
Oct4 reverse: 5′-GGCAGATGGTCGTTTGGCTGAATA-3′; 24 nt; 50% GC; Tm 70 °C
Optimal Tm: 71 °C
Sox2 forward: 5′-ATGGGCTCTGTGGTCAAGTC-3′; 20 nt: 55% GC; Tm 67 °C
Sox2 reverse: 5′-CCCTCCCAATTCCCTTGTAT-5′; 20 nt; 50% GC; Tm 64 °C
Optimal Tm: 65 °C
NANOG forward: 5′-GCTGAGATGCCTCACACGGAG-3′; 21 nt; 62% GC; Tm 71 °C
NANOG reverse: 5′-TCTGTTTCTTGACTGGGACCTTGTC-3′; 25 nt: 48%GC; Tm 69 °C
Optimal Tm: 70 °C

2.7 Statistical Analysis

Data regarding viability (0-100%) and growth or proliferation (630 nm absorbance) was measured by instrumentation and presented on the appropriate measurement scale. These data were imported into Microsoft Excel (Redmond, WA) and differences between experimental conditions were measured using two-tailed Student's t-tests, which are appropriate for parametric analysis of continuous data. Any statistically significant differences were verified using Analysis of Variance (ANOVA) due to the possibility of error involved with analysis of multiple two-way t-tests. Significance levels were set at alpha (□) = 0.05.

3. RESULTS

This retrospective analysis identified sixteen (n=16) cryopreserved DPSC isolates that were isolated between 2011 and 2015 (Table 1). Their baseline growth rates had been previously established, which was previously categorized as rapid doubling time or rDT (1-2 days), intermediate doubling time or iDT (4-6 days), or slow doubling time or sDT (10 - 14 days). Baseline viability prior to cryopreservation and upon thawing averaged 28.8%, ranging between 17.6% to 49.3%. Analysis of total RNA isolated from the DPSCs for mesenchymal stem cell (MSC) biomarkers revealed all DPSC isolates expressed Nestin, while the majority of DPSCs also expressed NANOG. Half of the DPSCs also continued to express both Sox-2 and Oct-4.

To evaluate the effect of alternative commercially available cell culture media on the growth and viability of DPSC isolates, all DPSC isolates were split between alpha-MEM and Dulbecco's Modified Eagle's Medium or DMEM, DMEM with Ham's F12 or DMEM:F12 and Roswell Park Memorial Institute or RPMI. For example, all DPSC isolates were cultured in alpha-MEM and viability was compared with DMEM (Fig. 1). This analysis revealed that although several DPSCs did not exhibit any significant change in viability (-3% to +3%), at least three DPSCs (dpsc-17322, dpsc-3882, dpsc-9894) exhibited significant decreases in cell viability, ranging from - 8% to -28%. In addition, at least six DPSCs exhibited significant increases in cell viability, ranging from +6% to +25% (dpsc-11836, dpsc-5423, dpsc-9765, dpsc-8604, dpsc-8124, dpsc-4595).

Next, all DPSC isolates were cultured in alpha-MEM and viability was compared with another commercially available media, DMEM:F12 (Fig. 2). This analysis revealed that although some

DPSCs did not exhibit any significant change in viability (-2% to +4%), at least eight DPSCs (dpsc-7089, dpsc-3924, dpsc-5423, dpsc-11418, dpsc-3882, dpsc-5423, dpsc-17322, dpsc-8604) exhibited significant decreases in cell viability, ranging from - 8% to -26%. Furthermore, at least five DPSCs exhibited significant increases in cell viability, ranging from +5% to +29% (dpsc-11836, dpsc-11750, dpsc-4595, dpsc-9765, dpsc-5653).

Finally, all DPSC isolates were cultured in alpha-MEM and viability was compared with the commercially available media RPMI (Fig. 3). This analysis revealed that although some DPSCs did not exhibit any significant change in viability (-2% to +2%), at least six DPSCs (dpsc-17322, dpsc-3882, dpsc-7089, dpsc-3924, dpsc-11418, dpsc-8604) exhibited significant decreases in cell viability, ranging from - 6% to -21%. Furthermore, at least five DPSCs exhibited significant increases in cell viability, ranging from +8% to +23% (dpsc-8124, dpsc-5423, dpsc-9765, dpsc-5653, dpsc-4595).

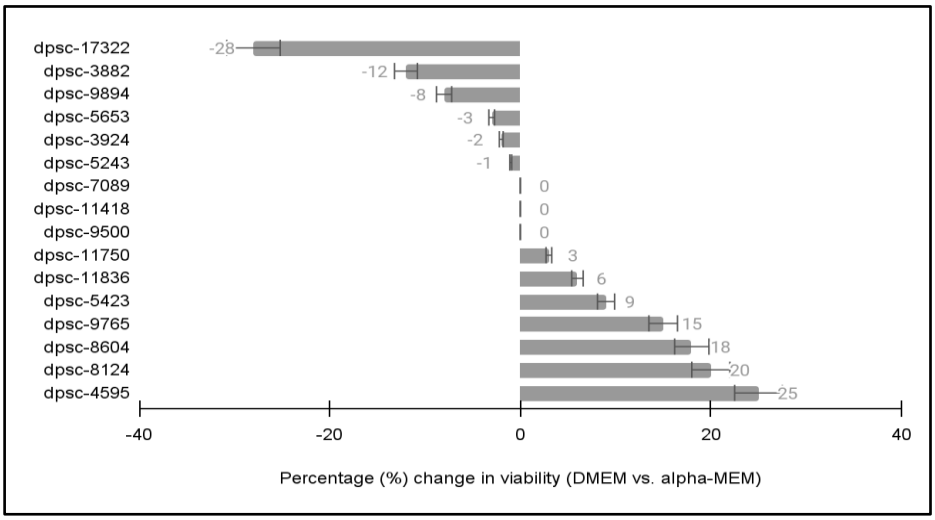

Fig. 1. Comparison of DPSC viability with alpha-MEM versus DMEM. Several DPSCs (n=7) did not exhibit any significant change in viability (-3% to +3%), but at least three (n=3) DPSCs exhibited decreased cell viability (-8% to -25%) and six (n=6) DPSCs exhibited increased cell viability (+6% to +25%)

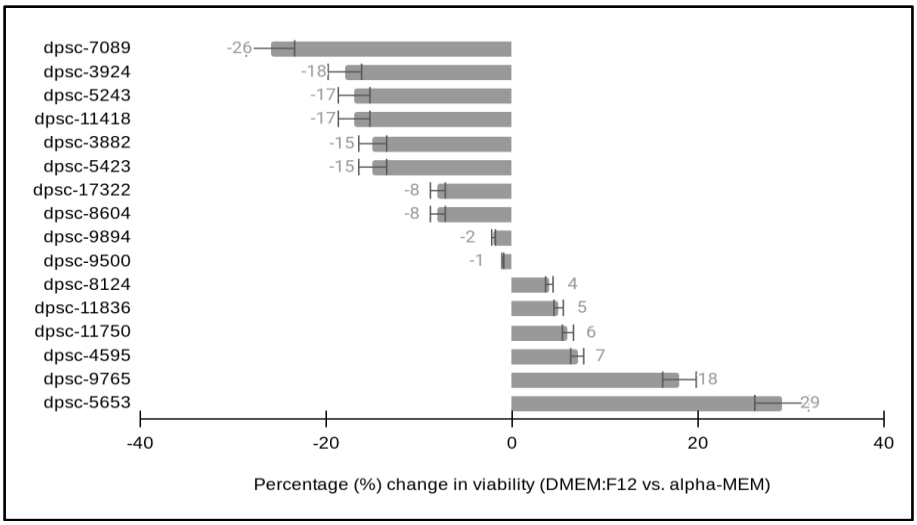

Fig. 2. Comparison of DPSC viability with alpha-MEM versus DMEM:F12. Some DPSCs (n=3) did not exhibit any significant change in viability (-2% to +4%), but at least three (n=8) DPSCs exhibited decreased cell viability (-8% to -26%) and five (n=5) DPSCs exhibited increased cell viability (+5% to +29%)

Table 1. Baseline characteristics of DPSC isolates

Year	Rate	Line	Days	Viability (%)	Sox-2	Oct-4	NANOG	Nestin
2011	slow (sDT)	dpsc-11750	13.1	37.9				Nestin
2011	slow (sDT)	dpsc-11836	12.9	24.7				Nestin
2011	slow (sDT)	dpsc-17322	10.6	39.1			NANOG	Nestin
2011	slow (sDT)	dpsc-11418	10.2	17.6				Nestin
2011	rapid (rDT)	dpsc-3924	1.9	21.6	Sox-2	Oct4	NANOG	Nestin
2012	rapid (rDT)	dpsc-5653	2.1	31	Sox-2	Oct4	NANOG	Nestin
2012	rapid (rDT)	dpsc-3882	2.6	31	Sox-2	Oct4	NANOG	Nestin
2012	rapid (rDT)	dpsc-7089	1.9	20.33	Sox-2	Oct4	NANOG	Nestin
2013	rapid (rDT)	dpsc-9765	2.3	25.9	Sox-2	Oct4	NANOG	Nestin
2013	slow (sDT)	dpsc-9500	10.4	20.7				Nestin
2013	intermediate (iDT)	dpsc-9894	5.1	25.6	Sox-2	Oct4	NANOG	Nestin
2014	rapid (rDT)	dpsc-5423	2.2	23.1	Sox-2	Oct4	NANOG	Nestin
2014	intermediate (iDT)	dpsc-8604	5.5	28.7	Sox-2	Oct4	NANOG	Nestin
2014	slow (sDT)	dpsc-8124	5.9	49.3			NANOG	Nestin
2015	slow (sDT)	dpsc-4595	11.2	30.7				Nestin
2015	intermediate (iDT)	dpsc-5243	4.2	33.7		Oct4		Nestin

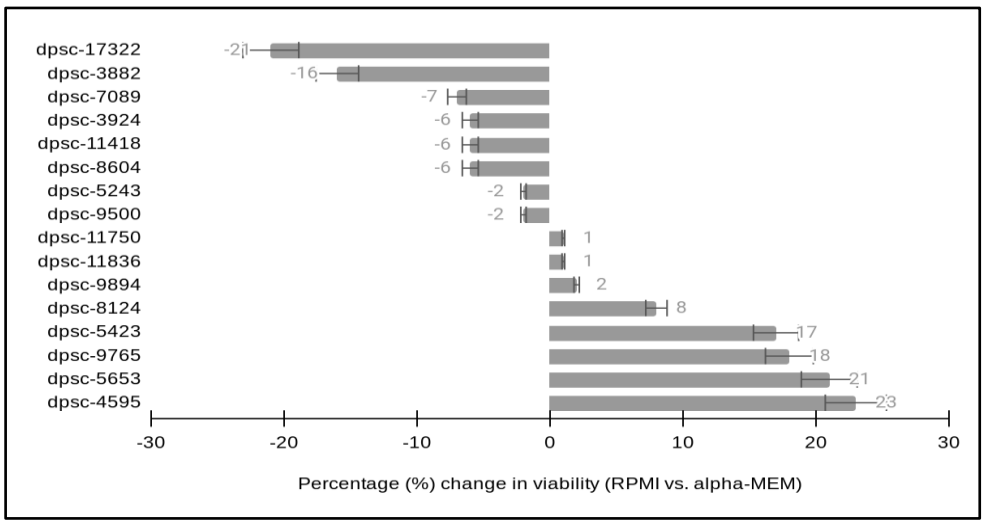

Fig. 3. Comparison of DPSC viability with alpha-MEM versus RPMI. Some DPSCs (n=5) did not exhibit any significant change in viability (-2% to +2%), but at least six (n=6) DPSCs exhibited decreased cell viability (-6% to -21%) and five (n=5) DPSCs exhibited increased cell viability (+8% to +23%)

Due to the observation that several DPSC isolates exhibited increased viability with one or more commercially available media alternatives, viability for all DPSCs in standard alpha-MEM media were plotted against the change in viability with the alternative media (DMEM, DMEM:F12, RPMI) (Fig. 4). This analysis demonstrated that for DPSCs with viability less than 50% (ranging between 28% and 49%), one or more alternative media substitutes induced significant increases in DPSC viability with only two minor exception (dpsc-5423 in DMEM:F12, dpsc-8604 in DMEM:F12 and RPMI). Interestingly, for DPSCs with viability greater than 50% (ranging between 50% and 66%), almost all of the alternative media substitutes induced significant decreases in DPSC viability with the exception of dpsc-8124, which increased viability in all three alternatives. However, no associations were found between viability, media responsiveness and DPSC biomarker expression.

4. DISCUSSION

The primary goal of this study was to evaluate any effects of the major, commercially available cell culture media (Dulbecco's Modified Eagle's Medium or DMEM, DMEM:F12, Roswell Park Memorial Institute or RPMI, and alpha-MEM) on DPSC phenotypes, such as growth and viability. The results of this study demonstrated that many DPSC isolates responded positively to one or more of these media, including DMEM, DMEM:F12, RPMI, - as well as the standard DPSC culture media alpha-MEM. These findings add significantly to the evidence regarding the potential culturing methods that may be routinely employed in various *ex vivo* and *in vitro* DPSC studies [17,20,35].

These findings also suggest that DPSC with viability lower than 50% in alpha-MEM may actually respond positively to alternative culture media, findings that support other studies exploring alternative media to determine if cell-specific responses could be observed [36-38]. Interestingly, these findings also demonstrated that DPSCs with viability greater than 50% using alpha-MEM may not respond necessarily positively to alternative media, suggesting some form of media testing for DPSC to separate those DPSC isolates with low viability may subsequently provide significant improvements in DPSC viability for future use - a goal of many recent studies and research efforts [39,40].

As more DPSC-related studies progress, standardized (and alternative) methods for DPSC culture and expansion are needed and any protocols or methods that provide significant improvement in DPSC viability or growth may prove invaluable [40-42]. Other recent studies have provided tips and tricks for improving stem cell viability, although many of the most recent techniques have involved methods to reduce or avoid the use of feeder layers and to improve feeder-free monocultures [43,44]. Since most of these major commercially available media

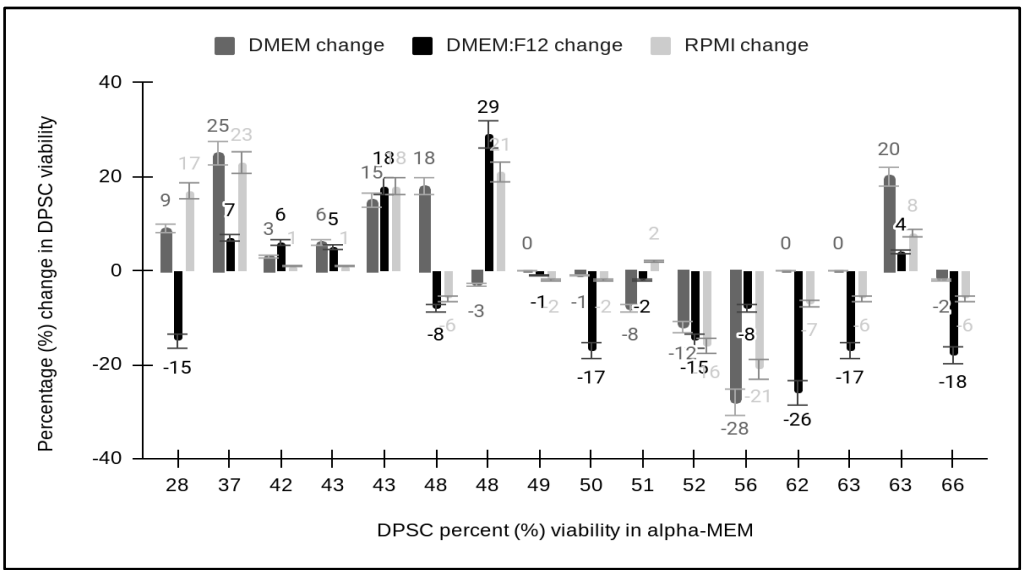

Fig. 4. Comparison of DPSC viability in alternative media plotted against viability in alpha-MEM. This analysis demonstrated that DPSCs with viability less than 50%, almost all of the alternative media induced increased viability. DPSCs with viability in alpha-MEM greater than 50% exhibited decreased DPSC viability in alternative media, with the exception of one DPSC (dpsc-8124)

contain similar constituents but at different ratios or concentrations, it will be necessary to further investigate these differences to determine the functional mechanisms associated with these observations.

For example, the most commonly used DPSC culturing media alpha-MEM contains minimal essential nutrients including glucose and amino acids - although it is also known to contain non-essential amino acids, sodium pyruvate, lipoic acid, vitamin B12, biotin, and ascorbic acid [45,46]. In contrast, DMEM has been developed to provide the minimum essential nutrients (glucose, amino acids and nucleotides) but has been modified to include higher levels of glutamine, glucose, sodium pyruvate and sodium bicarbonate to act as the most broadly suitable media for adherent cell culture - and has been recently demonstrated to improve cell viability and proliferative capacity in other stem cell models, which may be of particular relevance to DPSC isolates with lower viability [47-49]. Interestingly, some of the DPSCs responded to DMEM:F12, which was originally designed to facilitate hepatocyte and prostate epithelial cell growth using increased levels of choline, inositol, putrescine and specific amino acids and has recently been used to culture other types of stem cell isolates and may therefore be particularly useful for DPSC isolates, such as those identified with lower viability [50-52]. Finally, the response of some DPSCs to RMPI-1640, which contains biotin, vitamin B12, and PABA (not found in any other commercially available media), suggests that some metabolic pathways active within those DPSCs may respond preferentially to these components as has been observed in some studies of mesenchymal stromal cells and blood-derived stem cell progenitors [53,54].

Although these findings are significant and may help to improve viability and survival among DPSC isolates that are not responsive to traditional alpha-MEM media, there are some limitations of this study that should be considered. First, this is a retrospective study of previously isolated DPSCs that have been cryopreserved and thawed after significant time intervals. The responses of fresh and recently isolated DPSCs may be significantly different and it is hoped that future studies in this area will incorporate these parameters for further comparison and analysis. In addition, the financial and other limitations of this study did not allow for comparisons of larger groups of DPSCs from other research groups that may have been isolated from different areas, such as stem cells from exfoliated teeth (SHED), stem cells from the apical papilla (SCAP), periodontal ligament stem cells (PDLSC), and dental follicle stem cells (DFSC) [55]. As many research studies of this nature have explored DPSC phenotype and behavior in culture, future research in this area

might evaluate if these alternative media substitutions also provide increased viability and survival among these sub-populations of DPSCs [56,57].

5. CONCLUSIONS

The results of this study demonstrated that many DPSC isolates responded positively to one or more of these media, including DMEM, DMEM:F12, RPMI when viability was low using the standard DPSC culture media alpha-MEM. These findings may be broadly applicable and add significantly to the evidence regarding the potential culturing methods that may be employed in various *ex vivo* and *in vitro* DPSC studies These results strongly support the hypothesis that differential growth media screening may be necessary to ensure the highest viability and growth potential for all DPSC isolates.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by any producing company rather it was funded by personal efforts of the authors.

CONSENT

Informed consent was collected from all adult patients aged 18 years or older, with Parental Permission and Pediatric Assent collected from all parents or guardians, as well as pediatric patients under 18 years of age. Exclusion criteria included any patients or parents/guardians that declined to participate and any person not a UNLV SDM patient of record.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

ACKNOWLEDGEMENTS

The authors would like to acknowledge the presentation of preliminary data from this manuscript by GB and SS at the American Association for Dental Oral and Craniofacial Research (AADOCR) conference in 2022.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Al Madhoun A, Sindhu S, Haddad D, Atari M, Ahmad R, Al-Mulla F. Dental Pulp Stem Cells Derived From Adult Human Third Molar Tooth: A Brief Review. Front Cell Dev Biol. 2021;9:717624.
DOI: 10.3389/fcell.2021.717624
PMID: 34712658
PMCID: PMC8545885.
2. Liu J, Yu F, Sun Y, Jiang B, Zhang W, Yang J, Xu GT, Liang A, Liu S. Concise reviews: Characteristics and potential applications of human dental tissue-derived mesenchymal stem cells. Stem Cells. 2015;33(3):627-38.
DOI: 10.1002/stem.1909
PMID: 25447379.
3. Marrelli M, Codispoti B, Shelton RM, Scheven BA, Cooper PR, Tatullo M, Paduano F. Dental Pulp Stem Cell Mechanoresponsiveness: Effects of Mechanical Stimuli on Dental Pulp Stem Cell Behavior. Front Physiol. 2018 Nov 26;9:1685. doi: 10.3389/fphys.2018.01685.
PMID: 30534086; PMCID: PMC6275199.
4. Sui B, Wu D, Xiang L, Fu Y, Kou X, Shi S. Dental Pulp Stem Cells: From Discovery to Clinical Application. J Endod. 2020;46(9S):S46-S55.
DOI: 10.1016/j.joen.2020.06.027
PMID: 32950195
5. Tsutsui TW. Dental Pulp Stem Cells: Advances to Applications. Stem Cells Cloning. 2020 Feb 13;13:33-42.
DOI: 10.2147/SCCAA.S166759
PMID: 32104005
PMCID: PMC7025818
6. Piva E, Silva AF, Nör JE. Functionalized scaffolds to control dental pulp stem cell fate. J Endod. 2014 Apr;40(4 Suppl):S33-40.
DOI: 10.1016/j.joen.2014.01.013
PMID: 24698691
PMCID: PMC4034046.
7. Tian S, Wang J, Dong F, Du N, Li W, Song P, Liu Y. Concentrated Growth Factor Promotes Dental Pulp Cells Proliferation

and Mineralization and Facilitates Recovery of Dental Pulp Tissue. Med Sci Monit. 2019;25:10016-10028.
DOI: 10.12659/MSM.919316
PMID: 31877561
PMCID: PMC6944166

8. Hernández-Monjaraz B, Santiago-Osorio E, Monroy-García A, Ledesma-Martínez E, Mendoza-Núñez VM. Mesenchymal Stem Cells of Dental Origin for Inducing Tissue Regeneration in Periodontitis: A Mini-Review. Int J Mol Sci. 2018;19(4):944.
DOI: 10.3390/ijms19040944
PMID: 29565801
PMCID: PMC5979585
9. Aurrekoetxea M, Garcia-Gallastegui P, Irastorza I, Luzuriaga J, Uribe-Etxebarria V, Unda F, Ibarretxe G. Dental pulp stem cells as a multifaceted tool for bioengineering and the regeneration of craniomaxillofacial tissues. Front Physiol. 2015;6:289.
DOI: 10.3389/fphys.2015.00289
PMID: 26528190
PMCID: PMC4607862
10. Onizuka S, Iwata T. Application of Periodontal Ligament-Derived Multipotent Mesenchymal Stromal Cell Sheets for Periodontal Regeneration. Int J Mol Sci. 2019;20(11):2796.
DOI: 10.3390/ijms20112796
PMID: 31181666
PMCID: PMC6600219.
11. Amghar-Maach S, Gay-Escoda C, Sánchez-Garcés MÁ. Regeneration of periodontal bone defects with dental pulp stem cells grafting: Systematic Review. J Clin Exp Dent. 2019;11(4):e373-e381.
DOI: 10.4317/jced.55574.
PMID: 31110618
PMCID: PMC6522106
12. Mead B, Logan A, Berry M, Leadbeater W, Scheven BA. Concise Review: Dental Pulp Stem Cells: A Novel Cell Therapy for Retinal and Central Nervous System Repair. Stem Cells. 2017;35(1):61-67.
DOI: 10.1002/stem.2398
Epub 2016 Jun 16
PMID: 27273755.
13. Mead B, Berry M, Logan A, Scott RA, Leadbeater W, Scheven BA. Stem cell treatment of degenerative eye disease. Stem Cell Res. 2015;14(3):243-57.
DOI: 10.1016/j.scr.2015.02.003
Epub 2015 Feb 24
PMID: 25752437
PMCID: PMC4434205
14. Yang X, Li L, Xiao L, Zhang D. Recycle the dental fairy's package: overview of dental pulp stem cells. Stem Cell Res Ther. 2018;9(1):347.
DOI: 10.1186/s13287-018-1094-8
PMID: 30545418
PMCID: PMC6293656
15. Anitua E, Troya M, Zalduendo M. Progress in the use of dental pulp stem cells in regenerative medicine. Cytotherapy. 2018;20(4):479-498.
DOI: 10.1016/j.jcyt.2017.12.011
Epub 2018 Feb 12
PMID: 29449086.
16. Conde MC, Chisini LA, Grazioli G, Francia A, Carvalho RV, Alcázar JC, Tarquinio SB, Demarco FF. Does Cryopreservation Affect the Biological Properties of Stem Cells from Dental Tissues? A Systematic Review. Braz Dent J. 2016;27(6):633-640.
DOI: 10.1590/0103-6440201600980
PMID: 27982171.
17. Bakopoulou A, Apatzidou D, Aggelidou E, Gousopoulou E, Leyhausen G, Volk J, Kritis A, Koidis P, Geurtsen W. Isolation and prolonged expansion of oral mesenchymal stem cells under clinical-grade, GMP-compliant conditions differentially affects "stemness" properties. Stem Cell Res Ther. 2017;8(1):247.
DOI: 10.1186/s13287-017-0705-0
PMID: 29096714
PMCID: PMC5667471
18. 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(Suppl 1):S27-S36.
DOI: 10.4103/jpbs.JPBS_121_20
Epub 2020 Aug 28
PMID: 33149427
PMCID: PMC7595477
19. Couto de Carvalho LA, Tosta Dos Santos SL, Sacramento LV, de Almeida VR Júnior, de Aquino Xavier FC, Dos Santos JN, Gomes Henriques Leitão ÁC. Mesenchymal stem cell markers in periodontal tissues and periapical lesions. Acta Histochem. 2020;122(8):151636.
DOI: 10.1016/j.acthis.2020.151636
Epub 2020 Oct 22
PMID: 33132168
20. Bhandi S, Alkahtani A, Mashyakhy M, Ali Baeshen H, Mustafa M, Chohan H, Boreak N, Patil S. Study of optimal conditions for growth and osteogenic differentiation of dental pulp stem cells based on glucose

and serum content. Saudi J Biol Sci. 2021;28(11):6359-6364.
DOI: 10.1016/j.sjbs.2021.06.101
Epub 2021 Jul 6
PMID: 34759755
PMCID: PMC8568704

21. Li Z, Liu L, Wang L, Song D. The effects and potential applications of concentrated growth factor in dentin-pulp complex regeneration. Stem Cell Res Ther. 2021;12(1):357.
DOI: 10.1186/s13287-021-02446-y
PMID: 34147130
PMCID: PMC8214771
22. Oikonomopoulos A, van Deen WK, Manansala AR, Lacey PN, Tomakili TA, Ziman A, Hommes DW. Optimization of human mesenchymal stem cell manufacturing: the effects of animal/xeno-free media. Sci Rep. 2015;5:16570.
DOI: 10.1038/srep16570
PMID: 26564250
PMCID: PMC4643287
23. A Young, K Kingsley. Dental Pulp Stem Cells: A review of factors that influence the therapeutic potential of stem cell isolates. Biomaterials and Biomedical Engineering. 2015;2(2):61-69.
24. Tomlin A, Sanders MB, Kingsley K. The effects of cryopreservation on human dental pulp-derived mesenchymal stem cells. Biomaterials and Biomedical Engineering 2016;3(2):103-112.
Available:http://dx.doi.org/10.12989/bme.2016.3.2.102
25. Tomlin A, Nelson B, Kingsley. K. Dental Pulp Stem Cell Biomarkers for Cellular Viability Following Long-Term Cryopreservation. Int J Cell Sys Dev Biol. 2018;1(1):1-6.
26. Whiting M, Kingsley K. Expression of microRNA among dental pulp stem cell (DPSC) isolates. Current Research in Dentistry; 2019. [Online first]
27. Cinelli J, Mullins E, Kingsley K. Differential Effects of Bone Morphogenic Protein (BMP) and Vascular Endothelial Growth Factor (VEGF) on Dental Pulp Stem Cell (DPSC) Subpopulations. EC Dental Science. 2020;19(3):01-10.
28. Forgues C, Mullins E, Kingsley K. Effects of vascular endothelial growth factor (VEGF) on Dental Pulp Stem Cells (DPSC). Current Research in Medicine; 2019.
DOI: 10.3844/amjsp.2019
29. Agari K, Lin W, Kingsley K. Folic Acid-Modulated Growth of Dental Pulp Stem Cells (DPSCs). J Med Discov. 2018;3(3):jmd18024.
DOI: 10.24262/jmd.3.3.18024
30. K Loveland, A Young, M Khadiv, M Culpepper, K Kingsley. Dental Pulp Stem Cell (DPSC) Pluripotency Enhanced by Transforming Growth Factor (TGF-β1) *In vitro* may be inhibited by Differentiation-Inducing Factors Laminin-5 and Dexamethasone. International Journal of Biological Sciences and Applications. 2014;1(3):55-61. [Epub ahead of print]
31. Melling GE, Colombo JS, Avery SJ, Ayre WN, Evans SL, Waddington RJ, Sloan AJ. Liposomal Delivery of Demineralized Dentin Matrix for Dental Tissue Regeneration. Tissue Eng Part A. 2018;24(13-14):1057-1065.
DOI: 10.1089/ten.TEA.2017.0419
Epub 2018 Feb 21
PMID: 29316874
PMCID: PMC6033301.
32. Lee CP, Colombo JS, Ayre WN, Sloan AJ, Waddington RJ. Elucidating the cellular actions of demineralised dentine matrix extract on a clonal dental pulp stem cell population in orchestrating dental tissue repair. J Tissue Eng. 2015;6:2041731415586318.
DOI: 10.1177/2041731415586318
PMID: 26019808
PMCID: PMC4437905
33. Ferroni L, Gardin C, Sivolella S, Brunello G, Berengo M, Piattelli A, Bressan E, Zavan B. A hyaluronan-based scaffold for the in vitro construction of dental pulp-like tissue. Int J Mol Sci. 2015;16(3):4666-81.
DOI: 10.3390/ijms16034666
PMID: 25739081
PMCID: PMC4394441
34. Bae S, Kang B, Lee H, Luu H, Mullins E, Kingsley K. Characterization of Dental Pulp Stem Cell Responses to Functional Biomaterials Including Mineralized Trioxide Aggregates. J Funct Biomater. 2021 Feb 24;12(1):15.
DOI: 10.3390/jfb12010015
PMID: 33668171
PMCID: PMC8006251.
35. Smeda M, Galler KM, Woelflick M, Rosendahl A, Moehle C, Lenhardt B, Buchalla W, Widbiller M. Molecular Biological Comparison of Dental Pulp- and Apical Papilla-Derived Stem Cells. Int J Mol Sci. 2022;23(5):2615.

DOI: 10.3390/ijms23052615
PMID: 35269758
PMCID: PMC8910327

36. Ma S, Wang L, Zong B, Wang Y, Wang X, Shi Y, Yang Y, Chen Y. Cultivation of Hair Matrix Cells from Cashmere Goat Skins and Exemplified Applications. Animals (Basel). 2020 Aug 12;10(8):1400.
DOI: 10.3390/ani10081400
PMID: 32806500
PMCID: PMC7460477
37. Gonçalves LNC, Costa-Orlandi CB, Bila NM, Vaso CO, Da Silva RAM, Mendes-Giannini MJS, Taylor ML, Fusco-Almeida AM. Biofilm Formation by Histoplasma capsulatum in Different Culture Media and Oxygen Atmospheres. Front Microbiol. 2020;11:1455.
DOI: 10.3389/fmicb.2020.01455
PMID: 32754126
PMCID: PMC7365857
38. Boekema BK, Boekestijn B, Breederveld RS. Evaluation of saline, RPMI and DMEM/F12 for storage of split-thickness skin grafts. Burns. 2015;41(4):848-52.
DOI: 10.1016/j.burns.2014.10.016
Epub 2014 Nov 15
PMID: 25468477.
39. Madanagopal TT, Franco-Obregón A, Rosa V. Comparative study of xeno-free induction protocols for neural differentiation of human dental pulp stem cells in vitro. Arch Oral Biol. 2020;109:104572.
DOI: 10.1016/j.archoralbio.2019.104572
Epub 2019 Sep 25
PMID: 31600663
40. Di Scipio F, Sprio AE, Carere ME, Yang Z, Berta GN. A Simple Protocol to Isolate, Characterize, and Expand Dental Pulp Stem Cells. Methods Mol Biol. 2017;1553:1-13.
DOI: 10.1007/978-1-4939-6756-8_1
PMID: 28229403
41. Bakkar M, Liu Y, Fang D, Stegen C, Su X, Ramamoorthi M, Lin LC, Kawasaki T, Makhoul N, Pham H, Sumita Y, Tran SD. A Simplified and Systematic Method to Isolate, Culture, and Characterize Multiple Types of Human Dental Stem Cells from a Single Tooth. Methods Mol Biol. 2017;1553:191-207.
DOI: 10.1007/978-1-4939-6756-8_15
PMID: 28229417
42. Gronthos S, Arthur A, Bartold PM, Shi S. A method to isolate and culture expand human dental pulp stem cells. Methods Mol Biol. 2011;698:107-21.
DOI: 10.1007/978-1-60761-999-4_9
PMID: 21431514
43. Castro-Viñuelas R, Sanjurjo-Rodríguez C, Piñeiro-Ramil M, Rodríguez-Fernández S, López-Baltar I, Fuentes-Boquete I, Blanco FJ, Díaz-Prado S. Tips and tricks for successfully culturing and adapting human induced pluripotent stem cells. Mol Ther Methods Clin Dev. 2021;23:569-581.
DOI: 10.1016/j.omtm.2021.10.013
PMID: 34901305
PMCID: PMC8640166
44. Ye H, Wang Q. Efficient Generation of Non-Integration and Feeder-Free Induced Pluripotent Stem Cells from Human Peripheral Blood Cells by Sendai Virus. Cell Physiol Biochem. 2018;50(4):1318-1331.
DOI: 10.1159/000494589
Epub 2018 Oct 24
PMID: 30355953.
45. Rogulska O, Petrenko Y, Petrenko A. DMSO-free cryopreservation of adipose-derived mesenchymal stromal cells: expansion medium affects post-thaw survival. Cytotechnology. 2017;69(2):265-276.
DOI: 10.1007/s10616-016-0055-2.
Epub 2016 Dec 24
PMID: 28013442
PMCID: PMC5366964
46. Hata J, Tamura T, Yokoshima S, Yamashita S, Kabeno S, Matsumoto K, Onodera K. Chemically defined medium for the production of biologically active substances of CHO cells. Cytotechnology. 1992;10(1):9-14.
DOI: 10.1007/BF00376095
PMID: 1369106
47. Nakashima Y, Nahar S, Miyagi-Shiohira C, Kinjo T, Kobayashi N, Saitoh I, Watanabe M, Fujita J, Noguchi H. A Liquid Chromatography with Tandem Mass Spectrometry-Based Proteomic Analysis of Cells Cultured in DMEM 10% FBS and Chemically Defined Medium Using Human Adipose-Derived Mesenchymal Stem Cells. Int J Mol Sci. 2018;19(7):2042.
DOI: 10.3390/ijms19072042
PMID: 30011845
PMCID: PMC6073410.
48. Gat I, Maghen L, Filice M, Wyse B, Zohni K, Jarvi K, Lo KC, Gauthier Fisher A, Librach C. Optimal culture conditions are critical for efficient expansion of human testicular somatic and germ cells in vitro. Fertil Steril. 2017;107(3):595-605.e7.

DOI: 10.1016/j.fertnstert.2016.12.028
PMID: 28259258.

49. Joulai Veijouyeh S, Mashayekhi F, Yari A, Heidari F, Sajedi N, Moghani Ghoroghi F, Nobakht M. In vitro induction effect of 1,25(OH)2D3 on differentiation of hair follicle stem cell into keratinocyte. Biomed J. 2017;40(1):31-38.
DOI: 10.1016/j.bj.2016.08.007
Epub 2017 Mar 23
PMID: 28411880
PMCID: PMC6138590

50. Neal EH, Katdare KA, Shi Y, Marinelli NA, Hagerla KA, Lippmann ES. Influence of basal media composition on barrier fidelity within human pluripotent stem cell-derived blood-brain barrier models. J Neurochem. 2021;159(6):980-991.
DOI: 10.1111/jnc.15532
Epub 2021 Nov 18
PMID: 34716922
PMCID: PMC8688328

51. Nakashima Y, Omasa T. What Kind of Signaling Maintains Pluripotency and Viability in Human-Induced Pluripotent Stem Cells Cultured on Laminin-511 with Serum-Free Medium? Biores Open Access. 2016 Apr 1;5(1):84-93. doi: 10.1089/biores.2016.0001. PMID: 27096107; PMCID: PMC4834485.

52. Van Pham P, Truong NC, Le PT, Tran TD, Vu NB, Bui KH, Phan NK. Isolation and proliferation of umbilical cord tissue derived mesenchymal stem cells for clinical applications. Cell Tissue Bank. 2016;17(2):289-302.
DOI: 10.1007/s10561-015-9541-6
Epub 2015 Dec 17
PMID: 26679929.

53. Fekete N, Rojewski MT, Lotfi R, Schrezenmeier H. Essential components for ex vivo proliferation of mesenchymal stromal cells. Tissue Eng Part C Methods. 2014;20(2):129-39.
DOI: 10.1089/ten.TEC.2013.0061
Epub 2013 Jul 5
PMID: 23713576.

54. Ohta K, Yamane T, Koh KR, Ohta T, Nakamae H, Takubo T, Hino M, Tatsumi N. An effective method for recovering CD34 positive progenitor cells from peripheral blood stem cell apheresis products cryopreserved with simplified method. Osaka City Med J. 1999;45 (2):139-48.
PMID: 10730080.

55. Zhu SY, Yuan CY, Lin YF, Liu H, Yang YQ, Wong HM, Zhang CF, Wang PL, Gu M. Stem Cells from Human Exfoliated Deciduous Teeth (SHEDs) and Dental Pulp Stem Cells (DPSCs) Display a Similar Profile with Pericytes. Stem Cells Int. 2021;2021:8859902.
DOI: 10.1155/2021/8859902
PMID: 34349804
PMCID: PMC8328701

56. Patel M, Smith AJ, Sloan AJ, Smith G, Cooper PR. Phenotype and behaviour of dental pulp cells during expansion culture. Archives of Oral Biology. 2009;54(10):898-908.

57. Waddington RJ, Sloan AJ. Research advances in tissue regeneration by dental pulp stem cells. Tissue Engineering and Regeneration in Dentistry: Current Strategies. 2016:50-68.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/87039