Lasers in Medical Science (2019) 34:659–666
https://doi.org/10.1007/s10103-018-2637-z

ORIGINAL ARTICLE

Short-term evaluation of photobiomodulation therapy on the proliferation and undifferentiated status of dental pulp stem cells

L. S. Ferreira[1] · I. M. A. Diniz[2] · C. M. S. Maranduba[3] · S. P. H. Miyagi[4] · M. F. S. D. Rodrigues[5] · C. Moura-Netto[1] · Márcia Martins Marques[1,6]

Received: 16 March 2018 / Accepted: 10 September 2018 / Published online: 24 September 2018

Abstract

The aim of this in vitro study was to analyze the effect of photobiomodulation therapy (PBMT) on the proliferation and undifferentiating status of stem cell from human exfoliated deciduous teeth (SHEDs). PBMT was carried out with an aluminum gallium indium phosphide (InGaAlP) diode laser in contact and punctual mode (continuous wave, 660 nm, 20 mW, 0.028 cm^2, and average energy densities of 1 (1 s), 3 (4 s), 5 (7 s), 10 (14 s), 15 (21 s), or 20 (28 s) J/cm^2 per point). The immunoprofile of the SHEDs was analyzed using flow cytometry. Cell proliferation was assessed by the MTT reduction assay. Gene expressions of mesenchymal stem cell markers (*OCT4*, *Nestin*, *CD90*, and *CD105*) were assessed by RT-qPCR 48 h after PBMT. Data were compared by analysis of variance (ANOVA) and Tukey's test ($p \leq 0.05$). Cells cultured under nutritional deficit and treated with PBMT at 5 J/cm^2 presented similar cell growth than those of positive control group. Cell growth was significantly higher than those of other groups. Mesenchymal stem cell gene markers were still expressed after PBMT at 5 J/cm^2. In a short-term analysis, PBMT increases the number of stem cells with no interference in the undifferentiated state of the irradiated cells, which opens wide possibilities for application in tissue regeneration.

Keywords Photobiomodulation therapy · Mesenchymal stem cell · Cell proliferation · Gene expression · Stemness

Introduction

Stem cells are capable of self-renewal and differentiation into other types of cells [1]. Due to its undifferentiated nature, they can be stimulated or programmed to play specific biological functions, thus having key implications in the treatment of many diseases or in regenerative processes [2]. In the past few years, stem cell sources were found in adults, called adult stem cells, surpassing various ethical and religious barriers, which come along with the use of embryonic stem cells. Adult mesenchymal stem cells (MSCs) were isolated from almost every type of connective tissue, such as adipose [3], bone marrow tissues, periodontal ligament tissues [4], and dental pulp [5, 6]. Stem cells from dental pulp origin, from either permanent (dental pulp stem cells (DPSCs)) or deciduous teeth (stem cells from human exfoliated deciduous teeth (SHEDs)), are of great interest for tissue engineering and cell therapy purposes once they are easily harvested from dischargeable samples [7, 8]. Moreover, they can be obtained in reasonable quantities due to its great proliferation ability [9–11].

Despite all the potentialities of MSCs, the control of its viability, proliferation, and differentiation processes, before and/or following cell transplantation, is still a challenge in the tissue engineering field [12]. The passage of an in vitro to an in vivo system creates a stressful situation that can result in the loss of proliferative and differentiation potentials, or even the death of MSCs [13]. Photobiomodulation therapy

✉ Márcia Martins Marques
mmmarques@usp.br

1 Restorative Dentistry Department, School of Dentistry, Universidade de São Paulo, São Paulo, SP, Brazil

2 Restorative Dentistry Department, School of Dentistry, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

3 Institute of Biological Sciences, Federal University of Juiz de Fora, Juiz de Fora, MG, Brazil

4 School of Dentistry, Faculdades Metropolitanas Unidas (FMU), São Paulo, SP, Brazil

5 Biophotonics Applied to Health Science, University Nove de Julho, São Paulo, SP, Brazil

6 Departamento de Dentística, Faculdade de Odontologia, Universidade de São Paulo, Avenida Professor Lineu Prestes 2227, São Paulo, SP CEP 05508-000, Brazil

(PBMT), in turn, has demonstrated the ability to modulate cell growth, survival, and differentiation processes of many cell types [14–20] and could be used to overcome some of the drawbacks related to tissue engineering.

In fact, systematic reviews on the effects of PBMT on ddMSCs have shown that although no other clear conclusions were obtained due to the scarce number of publications, the results of these studies showed a tendency of PBMT to improve ddMSC viability and proliferation, especially when red laser wavelengths were used [21–23].

Studies on PBMT effects on MSCs have shown promising, but with controversial results that may be explained due to the great variability of the PBMT protocols [21–23]. The main challenge is based on applying the optimal amount of energy able to stimulate cell metabolism, without leading to phototoxicity [19]. Taken this into consideration, PBMT could be of importance for future tissue engineering or cell therapy, where the goal is to obtain large amount of stem cells with the maintenance of their undifferentiated status. Therefore, the aim of this in vitro study was to analyze the effect of PBMT on the proliferation and on the expression of a regular panel of mesenchymal stem cell markers in SHEDs grown under stressful condition.

Material and methods

Cell culture

The cells were obtained by using the explants plus enzymatic technique in human dental pulp fragments of two deciduous teeth extracted under local anesthesia due to advanced root resorption. The teeth were immersed into sterile saline containing antibiotics (penicillin and streptomycin 10,000 U/mL) solution and transported to the laboratory for processing. Within 2 h maximum, the dental pulp was removed with the help of a hypodermic needle under laminar flow. The tissue was washed twice in sterile calcium and magnesium-free phosphate-buffered saline (PBSA, pH 7.2) containing 4% antibiotic-antimicotic solution (Invitrogen, Carlsbad, CA, USA) and streptomycin (Invitrogen) and minced with the help of needles. Then, tissue fragments were immersed into a 0.25% trypsin solution (Invitrogen) and incubated at 37 °C for 15 min. After incubation, trypsin was inactivated using fetal bovine serum (FBS). Then, the explants were cultured in clonogenic medium [DMEM/Ham's F-12 culture media (1:1, Invitrogen), supplemented with 15% FBS (Hyclone, Logan, UT, USA), 100 U/mL penicillin (Invitrogen), 100 μg/mL streptomycin (Invitrogen), 2 mM L-glutamine (Invitrogen), and 2 mM non-essential amino acids (Invitrogen)]. Cells were maintained in an incubator at 37 °C in humid atmosphere containing 5% CO_2. The cells were passed every 4–5 days by washing the flasks twice in phosphate-buffered saline (PBS), followed by dissociating in a 0.25% trypsin solution. The cultures were passed when semiconfluent in order to prevent the differentiation of the cells by cell to cell contact. Cell culture medium was changed each 2 or 3 days, depending on the cell metabolism. Samples of the initial passages were cryopreserved in liquid nitrogen using dimethyl sulfoxide (DMSO; Sigma, St. Louis, MO, USA). All culture procedures were undertaken under laminar flow following the protocols of sterility of materials and solutions [24].

Immunophenotype profile

Cells up were expanded to the fifth passage for experimentation. Aliquots of SHEDs (1×10^6 cells/per antibody) were washed and resuspended in PBS containing saturating concentrations of conjugated primary monoclonal antibodies (1:200). The primary antibodies used were the following: embryonic markers OCT4 and Nanog (Santa Cruz Biotechnology, Santa Cruz, CA, USA), mesenchymal stem cell markers Nestin (Santa Cruz) and CD105 (Dako Corporation, Glostrup, Denmark), and hematopoietic markers CD31 and CD34 (both from Dako). Cells were sorted in a flow cytometer (FACS Calibur, BD Biosciences, San Jose, CA, USA) and a total of 10,000 events were analyzed using the 9.6.2 FlowJo Software Version (Tree Star, Ashland, OR, USA).

Stressful condition induction

Effects of PBMT may be observed on cultures grown under nutritional deficit due to its role in the oxidative processes ongoing in the cell, in particular in the respiratory chain of mitochondria [25]. This condition is obtained with the decrease in the percentages of FBS supplementation in the culture medium. Cells were seeded in 96-well plates (1×10^3 cell/well) in clonogenic medium (with 15% FBS). Six hours later, the culture medium was replaced by media with different FBS concentrations, as follows: 1%, 2.5%, 5%, 7.5%, 10%, and 15% (positive control). The cell viability was assessed by a 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) reduction assay after 24, 48, and 72 h. The absorbance data were used for the construction of cell growth curves.

Photobiomodulation therapy

PBMT was carried out with a continuous wave InGaAlP diode laser ($\lambda = 660$ nm, Photon Lase III, DMC-Equipamentos, SP, Brazil) (Fig. 1a) with a beam spot size of 0.028 cm^2, in punctual and contact mode, 20 mW power, and 0.714 W/cm^2 average power density through the

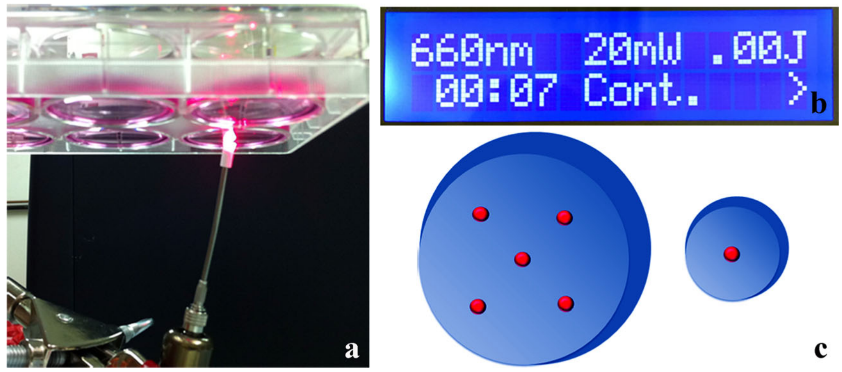

Fig. 1 Representative images of the PBMT protocol. **a** Photograph of the laser irradiation through the bottom of the cell culture plate. **b** The setup in the equipment display. **c** A diagram of the laser irradiation points in wells of the 6-well plate (9.5 cm^2 growth area; 5 points; 1 central and 4 equidistant) and a well of the 96-well plate (0.32 cm^2 growth area; 1 central point)

bottom of the culture wells (Fig. 1a). The choice of the parameters (Fig. 2b, Table 1), especially the wavelength, was based on previous studies [22]. In order to avoid laser overexposure, wells adjacent to each test well were kept empty. For the optically clear 96-well plates (Corning Inc., NY, US; proliferation assay), each well was irradiated in a single point; for 6-well plates (Corning; gene expression), each well was irradiated in five equidistant points (Fig. 1c). Irradiations were applied every other day during 1 week. The control groups were treated under identical conditions as the irradiated groups, however with the laser equipment turned off. Six different average energy densities were applied according to the experimental groups (Table 1). A power meter was used to check laser power output before and after irradiations.

Proliferation assay

Cells were seeded into 96-well plates (1×10^3 cell/well) in clonogenic medium (with 15% FBS) in alternate wells to prevent further over irradiation of the cells. Six hours later, the culture medium was replaced by deficient medium (defined in the first experiment) or clonogenic medium (positive control). Then, 12 h later, according to the experimental groups, the cultures were submitted or not to PBMT. The cell viability was assessed by the tetrazolium dye MTT [3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide] reduction assay at 24, 48, and 72 h after the last irradiation. The absorbance data were used for building the cell growth curves.

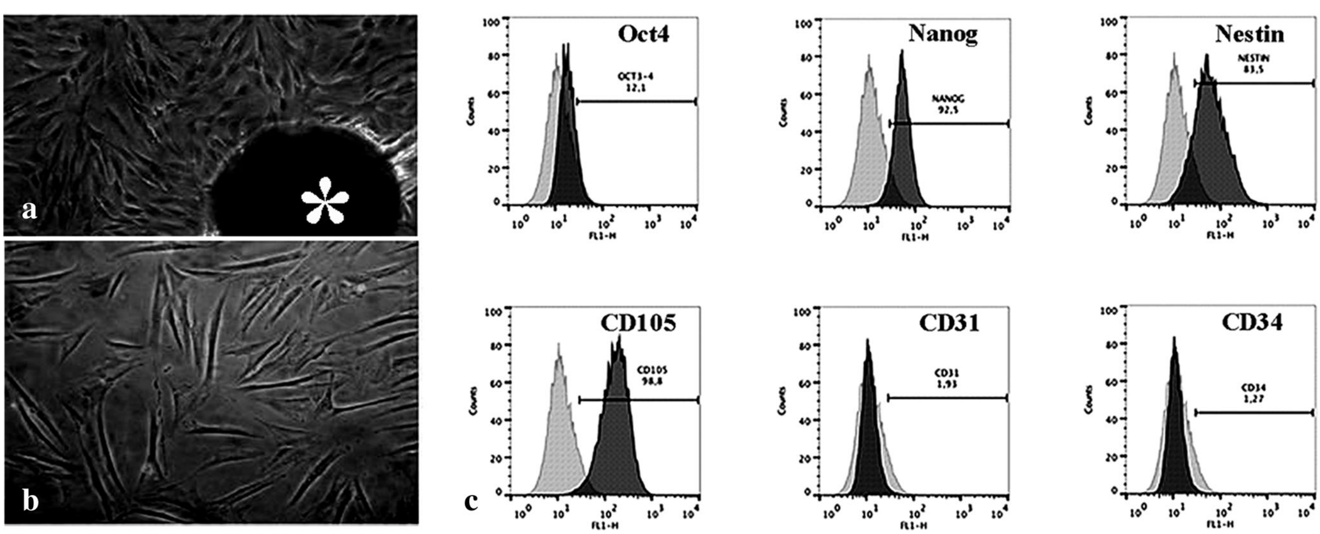

Fig. 2 Phase photomicrographs showing **a** an explant (asterisk—black sphere) with cells leaving the tissue and attaching to the bottom of the cell culture flask; **b** a subconfluent monolayer of cells (original magnification × 100); and **c** flow cytometry histograms representing the immunophenotype profile of SHEDs

Table 1 PBMT additional parameters according to the different experimental groups

Experimental groups	Culture medium	Average energy density (J/cm²)	Irradiation time per point (s)	Energy per point (J)
Positive control	Clonogenic medium (CM)	–	–	–
Negative control	Nutritional deficit (NDM)	–	–	–
L1	NDM	1	1	0.02
L3	NDM	3	4	0.08
L5	NDM	5	7	0.14
L10	NDM	10	14	0.28
L15	NDM	15	21	0.42
L20	NDM	20	28	0.56

Gene expression

The gene expression was observed by the real-time quantitative polymerase chain reaction (RT-qPCR). Briefly, the cells (5×10^4 cells/well) were seeded into 6-well plates ($n = 3$). Six hours later, the medium was replaced by the nutritional deficient medium (defined in the first experiment) or fresh medium (positive control). Then, 12 h later, PBMT was applied only in two experimental groups: L5 (5 J/cm^2) and L20 (20 J/cm^2), corresponding to the smallest average energy density with positive effects on cell proliferation and the highest average energy density applied in the proliferation assay. Total RNA from the cells was extracted 48 h later using RNEasy Mini Kit (Qiagen, Cat#74104, Valencia, CA, USA), following the manufacturer's instructions and treated with DNase I (Invitrogen). The cDNA templates were obtained by reverse transcription of 1 μ of total RNA using the High Capacity cDNA Archive kit (Applied Biosystems, Foster City, CA, USA). The products were subjected to qPCR analysis with SYBR Green dye 1 (SYBR Green Master Mix®, Applied Biosystems) and specific primers to *OCT4*, *Nestin*, *CD90*, and *CD105* genes as well as to constitutive gene *GAPDH* (Table 2). The thermal cycling was carried out by Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems), starting with 95 °C for 10 min hold, followed by 40 amplification cycles of 95 °C for 10 s and 58–62 °C for 1 min. Dissociation curve analyses of all qPCR reactions were performed at the end of cycling to verify the specificity of PCR product. The quantitative error of all triplicate samples was < 10%. PCR efficiencies were obtained from fivefold serial dilutions of cDNA templates quantified in triplicates.

Statistical analysis

All experiments were repeated at least three times. Data obtained from the cell proliferation assay and gene expression were compared using ANOVA followed by Tukey's post hoc test. For all data analysis, statistical significance level was set at 5%. All tests were performed using the program GraphPad Prism 5.0 (GraphPad Software, CA, EUA).

Results

Stem cells from exfoliated deciduous teeth were successfully obtained by the explant technique (Fig. 2a, b). The

Table 2 Primer sequences of the genes studied and characteristics of the reactions

	Gene	Sequence 5′ → 3′	Fragment (bp)	AT (°C)
Control	*GAPDH F* *GAPDH R*	GAAGGTGAAGGTCGGAGTC GAAGATGGTGATGGGATTTC	226	58
Embryonic markers	*OCT4 F* *OCT4 R*	ACTTCACTGCACTGTACTCCTCAG AGGTTCTCTTTCCCTAGCTCCTC	158	60
Mesenchymal markers	*Nestin F* *Nestin R*	GACCACTCCAGTTTAGAGGCTAAG GAATCTCCTCTCCCAGAGACTTC	244	60
	CD90 F *CD90 R*	CTAGTGGACCAGAGCCTTCG TGGAGTGCACACGTGTAGGT	236	60
	CD105 F *CD105 R*	TGCCACTGGACACAGGATAA CCTTCGAGACCTGGCTAGTG	205	62

AT annealing temperature, *bp* base pairs

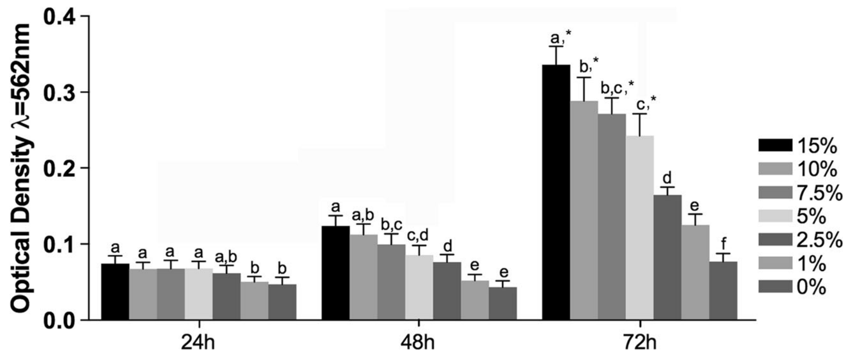

Fig. 3 Cell growth under different FBS concentrations (nutritional deficit)

lineage was isolated, identified, and characterized by flow cytometry (Fig. 2c). Cells were positive for the embryonic (OCT4 and Nanog) and mesenchymal stem cell (Nestin and CD105) markers and negative for the hematopoietic stem cell markers (CD31 and CD34).

Determination of fetal bovine serum concentration

Figure 3 illustrates cell growth under different concentrations of FBS. Significant increase in cell viability was observed in groups grown with 5% FBS and up ($p < 0.01$). Cells grown at the concentration of 5% FBS presented a continuous and exponential growth, but at a significantly lower rate than that of cell grown under ideal FBS supplementation (15% FBS-positive control) ($p < 0.05$). Thus, the concentration of 5% was chosen to verify the PBMT effect on the clonogenicity of SHEDs.

Effect of PBMT on cell proliferation

Negative control (5% FBS-non-irradiated) showed growth rate smaller than that of positive control (15% FBS) (Fig. 4). Cells grown under nutritional deficit (5% FBS) and irradiated with all parameters presented similar growth rate than negative control cells, except by the group irradiated with 5 J/cm^2 (L5). Cells grown at 5% FBS and treated with PBMT at 5 J/cm^2 exhibited growth significantly higher than negative control cells and similar to that of non-irradiated positive control cells grown under ideal conditions ($p < 0.05$).

Effects of PBMT on gene expression

Figure 5 depicts the gene expression profile of SHEDs cultured under nutritional deficit and irradiated at 5 or 20 J/cm^2. The mRNA expression levels of *OCT4*, *Nestin*, and *CD90* genes were higher in the 5 J/cm^2 irradiated group (L5) than all other groups ($p < 0.05$). The expression of the *CD105* was significantly smaller in the L5 groups than all other groups ($p < 0.05$). The *CD105* gene was upregulated in cells of the negative control and L20 (20 J/cm^2). At 20 J/cm^2 groups, all genes were expressed but only *OCT4* and *CD105* were upregulated when compared to control group grown at 15% FBS ($p < 0.05$). At 5% FBS, the expressions of *Nestin* and *CD90* were downregulated in relation to control group grown at 15% FBS ($p < 0.05$).

Discussion

An important source of adult stem cells is the dental pulp tissue, which, as such as the bone marrow, contains some undifferentiated cells within an already differentiated tissue [5]. It is widely accepted that in a MSC population, only a proportion of cells satisfy the typical MSC criteria in a single cell level, while the others are already committed. Once isolated, despite their high clonogenicity and tripotency, these cells may partially lose potential after in vivo transplantation, leading to an important drawback in tissue engineering [12, 26, 27]. In this context, PBMT could improve cell viability in an overwhelming microenvironment, possibly leading to

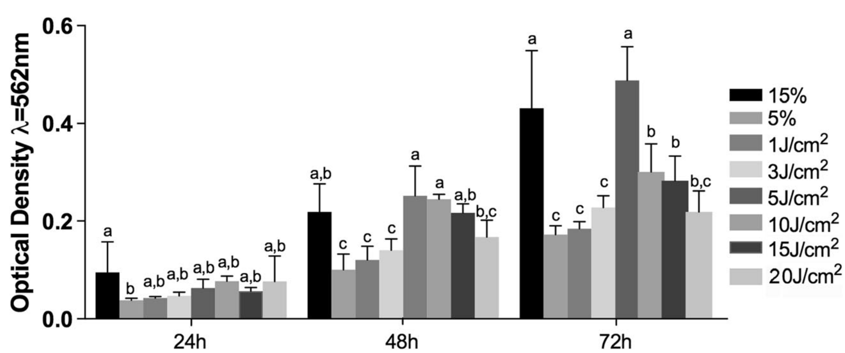

Fig. 4 Cell growth of all experimental groups. *Significantly higher than all other groups

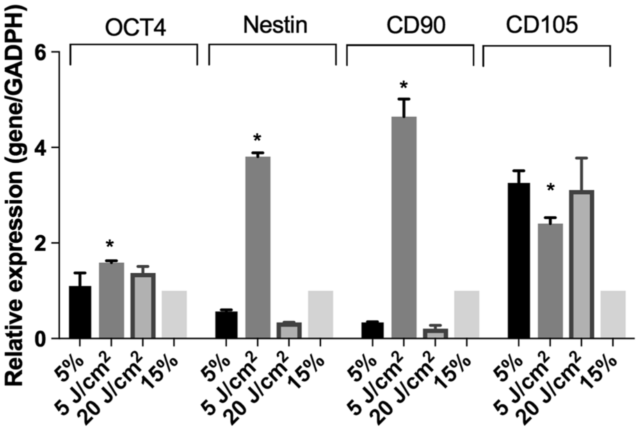

Fig. 5 Relative gene expression profile of SHEDs cultured under nutritional deficit. Controls and SHEDs irradiated at 5 or 20 J/cm^2

faster tissue neoformation. Thus, in this study, we aimed to evaluate the most appropriate PBMT parameters able to stimulate stem cell proliferation growth under nutritional deficit, while assessing any gene expression change in a commonly used mesenchymal stem cell marker panel.

Markers of stemness are molecular signatures that distinguish stem cells from other differentiated cell types in the body. In fact, the expression of stem cell markers is tissue and developmental stage dependent and multiple stem cell markers should be used in combination to define true stem cell populations in a tissue [28]. Thus, to identify the source and to confirm the stemness nature of our lineage, the immunophenotype profile of the cells was checked by using an antibody panel. Our cells were positive for the embryonic (OCT-4 and Nanog) and mesenchymal stem cell markers (Nestin and CD105) and negative for the hematopoietic stem cell markers (CD31, CD34), confirming previous data [5, 29, 30].

PBMT works through mitochondria molecules' excitation, such as cytochrome c oxidase (cyt c), in the region of red or near red (600–1000 nm) spectrum. As such, cyt c in its intermediate forms (i.e., under a condition of oxidative stress) is considered one of the primary photoaceptors of PBMT [31]. When excited, cyt c provides higher rate of pumping of hydrogen protons into the mitochondrial inner space, generating energy and, therefore, reactive oxygen species (ROS). ROS can modulate a wide range of cellular processes, but their concentrations are tightly regulated by potent antioxidant enzymes to prevent inadvertent damage [32].

To prevent cell death due to ROS imbalance and phototoxicity, a maximum dose threshold for PBMT should be determined to each cell type [19]. Accordingly, to assess the most appropriate dose parameter applied on SHEDs, we reproduced an oxidative stress scenario in vitro by decreasing the concentration of fetal bovine serum in the cell culture medium [14–17, 20]. Thus, we performed a proliferative assay to detect the FBS concentration not sufficient to prevent cell growth, but rather sufficient to make it significantly lower than controls grown under optimal nutrition conditions (15% FBS). We found that the culture medium supplemented with 5% FBS fulfilled this condition and was chosen as the FBS concentration for the following experiments.

To finally determine the maximum dose threshold to be applied on SHEDs, cells were cultured under nutritional deficit (5% FBS) and irradiated at 1, 3, 5, 10, 15, or 20 J/cm^2. Following irradiation at 5 J/cm^2, cells exhibited similar growth of non-irradiated cells grown under ideal conditions (at 15% FBS), regardless the experimental time interval evaluated. These results are in agreement with previous studies from our research group and with current literature [20–23].

The expression of stemness genes controls the establishment, survival, and maintenance of stem cells in their undifferentiated states. In fact, many growth factors and signaling molecules in the body regulate the specific gene expression pattern and some of them might be altered by photobiomodulation. Thus, the stemness gene expression patterns of the SHEDs studied here were reassessed by RT-qPCR after PBMT. All the four genes evaluated (*OCT-4*, *Nestin*, *CD90*, and *CD105*) were still expressed in SHEDs after cultivation at 5% FBS and irradiation at 5 J/cm^2, corroborating with the initial characterization status of the cells assessed by immunofluorescence. It is noteworthy that the expression of reprogramming markers, such as OCT-4, may be detectable at the beginning of ddMSC passages or in more immature cells such as the embryonic stem cells. However, sequential loss of this marker present in the cell nucleus during culture may occur and may be related to the fate of ddMSCs will take [30].

Current studies in PBMT have been focused on stem cell differentiation potential under several stimuli, and little is known about the effects of PBMT on the undifferentiated status of these cells after stimulation. A remarkable report on the effects of photobiomodulation and its mechanism of action on latent TGFβ—a growth factor that modulates pluripotency and differentiation of stem cells—observed a decrease in the expression of stem cell markers after PBMT, such as STRO-1, CD90, CD117, and CD44, suggesting that PBMT may determine the dental pulp cells' fate by directing their differentiation (i.e., taking stem cells far away from their initial undifferentiated status) [33]. These results do not corroborate with our findings. Nevertheless, it is not clear in which time point the authors performed this analysis and whether mineral induction was being performed or not.

We concluded that PBMT at 5 J/cm^2 is able to improve cell proliferation in an oxidative stress milieu without interfering with the undifferentiated status of MSCs in cultures under low passages in a short-term evaluation period. Thus, PBMT may be a promise tool to improve MSCs' self-renewal and survival during stem cell transplantation.

Funding information LSF and CNN were supported with scholarship from Fapesp # 2008/04708-0 and 2009/13483-4, respectively. MMM was supported by CNPq #307874/2014-1 and Fapesp #2009/11644.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was approved by a Local Research Ethics Committee (process number 166/2009). Patients provide their signed written informed consent.

Abbreviations *ANOVA,* Analysis of variance; *AT,* Annealing temperature; *bp,* Base pairs; *CM,* Clonogenic medium; *ddMSCs,* Dental-derived mesenchymal stem cells; *DMSO,* Dimethyl sulfoxide; *DPSCs,* Dental pulp stem cells; *FBS,* Fetal bovine serum; *InGaAlP,* Aluminum gallium indium phosphide; *MSCs,* Mesenchymal stem cells; *MTT,* 3-4,5-Dimethylthiazol-2-yl:-2,5-diphenyltetrazolium bromide; *NDM,* Nutritional deficit; *PBMT,* Photobiomodulation therapy; *PBSA,* Calcium and magnesium-free phosphate-buffered saline; *ROS,* Reactive oxygen species; *RT-qPCR,* Real-time quantitative polymerase chain reaction; *SHEDs,* Human exfoliated deciduous teeth; *SHEDs,* Stem cells from human exfoliated deciduous teeth

References

1. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Dj P, Horwitz E (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8(4):315–317
2. Borowski M, Stein GS (2011) Introduction to pluripotent stem cells: biology and applications. In: Stein GS, Borowski MA, Luong MX, Shi MJ, Smith KP, Vasquez P (eds) Human stem cell technology and biology. Willey-Blackwell, Hoboken, pp 3–7
3. Mvula B, Mathope T, Moore T, Abrahamse H (2008) The effect of low level laser irradiation on adult human adipose derived stem cells. Lasers Med Sci 23(3):277–282
4. Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, Young M, Robey PG, Wang CY, Shi S (2004) Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 364(9429):149–155
5. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S (2000) Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A 97(25):13625–13630
6. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S (2003) SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A 100(10):5807–5812
7. Yan X, Qin H, Qu C, Tuan RS, Shi S, Huang GT-J (2010) iPS cells reprogrammed from human mesenchymal-like stem/progenitor cells of dental tissue origin. Stem Cells Dev 19(4):469–480
8. Gronthos S (2011) The therapeutic potential of dental pulp cells: more than pulp fiction? Cytotherapy 13(10):1162–1163
9. Huang GT, Gronthos S, Shi S (2009) Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. J Dent Res 88(9):792–806
10. Kawashima N (2012) Characterisation of dental pulp stem cells: a new horizon for tissue regeneration? Arch Oral Biol 57(11):1439–1458
11. Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, Boyde A et al (2002) Stem cell properties of human dental pulp stem cells. J Dent Res 81(8):531–535
12. Xinaris C, Morigi M, Benedetti V, Imberti B, Fabricio AS, Squarcina E, Benigni A, Gagliardini E, Remuzzi G (2013) A novel strategy to enhance mesenchymal stem cell migration capacity and promote tissue repair in an injury specific fashion. Cell Transplant 22(3):423–436
13. Suchánek J, Soukup T, Ivancaková R, Karbanová J, Hubková V, Pytlík R, Kucerová L (2007) Human dental pulp stem cells—isolation and long term cultivation. Acta Med (Hradec Kralove) 50(3): 195–201
14. Marques MM, Pereira AN, Fujihara NA, Nogueira FN, Eduardo CP (2004) Effect of low-power laser irradiation on protein synthesis and ultrastructure of human gingival fibroblasts. Lasers Surg Med 34(3):260–265
15. Fujihara NA, Hiraki KR, Marques MM (2006) Irradiation at 780 nm increases proliferation rate of osteoblasts independently of dexamethasone presence. Lasers Surg Med 38(4):332–336
16. Eduardo FP, Mehnert DU, Monezi TA, Zezell DM, Schubert MM, Eduardo CP, Marques MM (2007) Cultured epithelial cells response to phototherapy with low intensity laser. Lasers Surg Med 39(4):365–372
17. Eduardo F de P, Bueno DF, de Freitas PM, Marques MM, Passos-Bueno MR, Eduardo Cde P, Zatz M (2008) Stem cell proliferation under low intensity laser irradiation: a preliminary study. Lasers Surg Med 40(6):433–438
18. Hou J, Zhang H, Yuan X, Li J, Wei Y, Hu S (2008) In vitro effects of low-level laser irradiation for bone marrow mesenchymal stem cells: proliferation, growth factors secretion and myogenic differentiation. Lasers Surg Med 40(10):726–733
19. Engel KW, Khan I, Arany PR (2016) Cell lineage responses to photobiomodulation therapy. J Biophotonics 9(11–12):1148–1156
20. Diniz IMA, Carreira ACO, Sipert CR, Uehara CM, Moreira MSN, Freire L, Pelissari C, Kossugue PM, de Araújo DR, Sogayar MC, Marques MM (2018) Photobiomodulation of mesenchymal stem cells encapsulated in an injectablerhBMP4-loaded hydrogel directs hard tissue bioengineering. J Cell Physiol 233(6):4907–4918
21. Borzabadi-Farahani A (2016) Effect of low-level laser irradiation on proliferation of human dental mesenchymal stem cells; a systemic review. J Photochem Photobiol B 162:577–582
22. Marques MM, Diniz IM, de Cara SP, Pedroni AC, Abe GL, D'Almeida-Couto RS, Lima PL, Tedesco TK, Moreira MS (2016) Photobiomodulation of dental derived mesenchymal stem cells: a systematic review. Photomed Laser Surg 34(11):500–508
23. Staffoli S, Romeo U, Amorim RNS, Migliau G, Palaia G, Resende L, Polimeni A (2017) The effects of low level laser irradiation on proliferation of human dental pulp: a narrative review. Clin Ter 168(5):e320–e326
24. Freshney RI (2000) Biology of the cultured cell. In: Culture of animal cells: a manual of basic technique (4 ed). Willey, New York, 577p
25. Karu T (1989) Laser biostimulation: a photobiological phenomenon. J Photochem Photobiol B 3:638–640
26. Mimeault M, Batra SK (2009) Aging of tissue-resident adult stem/progenitor cells and their pathological consequences. Panminerva Med 51:57–79

27. Wagner W, Bork S, Horn P, Krunic D, Walenda T, Diehlmann A, Benes V, Blake J, Huber FX, Eckstein V, Boukamp P, Ho AD (2009) Aging and replicative senescence have related effects on human stem and progenitor cells. PLoS One 4: e5846
28. Lv FJ, Tuan RS, Cheung KM, Leung VY (2014) Concise review: the surface markers and identity of human mesenchymal stem cells. Stem Cells 32(6):1408–1419
29. Kerkis I, Kerkis A, Dozortsev D, Stukart-Parsons GC, Gomes Massironi SM, Pereira LV, Caplan AI, Cerruti HF (2006) Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers. Cells Tissues Organs 184(3–4):105–116
30. Liu L, Wei X, Ling J, Wu L, Xiao Y (2011) Expression pattern of Oct-4, Sox2, and c-Myc in the primary culture of human dental pulp derived cells. J Endod 37(4):466–472
31. Karu T (1999) Primary and secondary mechanisms of action of visible to near-IR radiation on cells. J Photochem Photobiol B 49(1):1–17
32. Thannickal VJ, Fanburg BL (2000) Reactive oxygen species in cell signaling. Am J Phys Lung Cell Mol Phys 279(6): L1005–L1028
33. Arany PR, Cho A, Hunt TD, Sidhu G, Shin K, Hahm E et al (2014) Photoactivation of endogenous latent transforming growth factor-β1 directs dental stem cell differentiation for regeneration. Sci Transl Med 6(238):238ra69