Lasers Med Sci (2016) 31:973–978
DOI 10.1007/s10103-016-1945-4

ORIGINAL ARTICLE

Low-level laser therapy in 3D cell culture model using gingival fibroblasts

Fernanda G. Basso[1] · **Diana G. Soares**[1] · **Carlos Alberto de Souza Costa**[1] · **Josimeri Hebling**[1]

Received: 25 January 2016 / Accepted: 20 April 2016 / Published online: 28 April 2016

Abstract Besides extensive data about the effects of low-level laser therapy (LLLT) on different cell types, so far, these results were obtained from monolayer cell culture models, which have limitations in terms of cell morphology and phenotype expression. Therefore, for better in vitro evaluation of the effects of LLLT, this study was performed with a 3D cell culture model, where gingival fibroblasts were seeded in collagen matrix. Cells isolated from a healthy patient were seeded in wells of 24-well plates with culture medium (DMEM) supplemented with 10 % fetal bovine serum and collagen type I solution. After 5 days, a serum-free DMEM was added to the matrices with cells that were subjected or not to three consecutive irradiations of LLLT by means of the LaserTABLE diode device (780 nm, 25 mW) at 0.5, 1.5, and 3 J/cm^2. Twenty-four hours after the last irradiation, cell viability and morphology as well as gene expression of growth factors were assessed. Histological evaluation of matrices demonstrated uniform distribution and morphology of gingival fibroblasts within the collagen matrix. LLLT at 3 J/cm^2 increased gingival fibroblast viability. Enhanced gene expression of hCOL-I and hEGF was observed for 0.5 J/cm^2, while no significant changes were detected for the other irradiation densities tested. In conclusion, LLLT promoted biostimulation of gingival fibroblasts seeded in a 3D cell culture model, demonstrating that this model can be applied for phototherapy studies and that LLLT could penetrate the collagen matrix to increase cell functions related to tissue repair.

✉ Carlos Alberto de Souza Costa
casouzac@foar.unesp.br

[1] Araraquara School of Dentistry, University Estadual Paulista (UNESP), R Humaitá, 1680, 14801-903 Araraquara, SP, Brazil

Keywords Low-level laser therapy · Gingival fibroblasts · Cell culture

Introduction

Low-level laser therapy (LLLT) has been used for the treatment of several medical conditions, such as muscle skeletal disorders and pain [1, 2] as well as ulcerous conditions [3]. In dentistry, a number of studies have demonstrated that LLLT is suitable to treat different oral diseases, such as aphthous ulcers [4], oral mucositis [5], salivary pathological conditions [6], and herpetic lesions [7, 8]. Clinical studies have also shown that specific laser therapies are capable of reducing inflammatory reaction and pain symptomatology, promoting faster tissue healing [5, 9].

To elucidate the effects of LLLT at cellular and molecular levels and to establish parameters of irradiation for oral mucosa cells, several in vitro studies have been performed with cells isolated from oral mucosal tissues. Most of these investigations showed that this therapy increases cellular proliferation and migration, enhancing the expression and synthesis of growth factors and extracellular proteins as well as modulating gene expression of inflammatory mediators [10–15]. However, these studies were designed for monolayer cell culture models, in which some cell functions can be inhibited or diminished [16, 17]. In fact, the use of the monolayer cell culture model may not fully reproduce the LLLT interaction with oral mucosal cells, considering the necessity of light penetration through different tissues during irradiation [18, 19].

Previous investigations demonstrated that 3D cell culture models provide a better environment for cell morphology and phenotype analysis, allowing cells to migrate, establishing communication among them and with the extracellular matrix [9, 10]. It seems, then, that more credible data concerning cellular responses to different stimuli, such as LLLT, may be obtained from

the 3D culture models [20–23]. Consequently, the main objective of the present study was to evaluate the suitability of a specific 3D culture model for assessing the effects of LLLT on oral mucosal cells.

Materials and methods

Cell isolation

Primary gingival fibroblasts were obtained from gingival tissue of a healthy young patient undergoing tooth extraction, according to a protocol approved by the Ethics Committee of the Araraquara School of Dentistry (CAAE: 14342113.7.0000.5416), and after the patient's agreement.

The gingival fragment was placed in a sterilized 15-ml Falcon tube containing 5 ml of culture medium (Dulbecco's modified Eagle medium [DMEM], Gibco, Carlsbad, CA, USA) supplemented with antibiotic/antimycotic solutions (streptomycin and fungizone; Gibco) and transported to the Laboratory of Experimental Pathology and Biomaterials (LEPB). For cell isolation, the gingival fragment was cut into small parts that were placed in a sterilized petri dish containing 3 ml of DMEM and type I collagenase (3 mg/ml; Worthington Biochemical Corp., Lakewood, NJ, USA) and incubated at 37 °C for 24 h. After this, culture medium containing cells and tissue debris was transferred to a tube and centrifuged (4000×*g*). The cellular pellet was suspended in fresh culture medium supplemented with 10 % of fetal bovine serum (FBS) and transferred to 75-cm^2 sterilized cell culture flasks. Culture medium was replaced every 48 h until 80 % confluence was reached. At this point, cells were subcultured with 5 ml of 0.25 % trypsin solution (Gibco).

3D cell culture model

For the 3D cell culture model, gingival fibroblasts were seeded in a collagen matrix as previously described [24].

First, wells of 24-well plates were individually covered with an acellular matrix composed of DMEM containing 10 % FBS and type I collagen (BD Biosciences, Franklin Lakes, NJ, USA) at a final concentration of 0.77 mg/ml. The plates were then maintained in a laminar flow hood at room temperature for 30 min. After that, gingival fibroblasts were detached with 0.25 % trypsin (Gibco), and a cellular matrix of DMEM, type I collagen (0.77 mg/ml), and fibroblasts at 1×10^5 cells/ml was applied to the top of the acellular matrix, which was maintained in the laminar flow hood at room temperature for 1 h, followed by incubation for an additional 1 h. After this period, a supplemental volume of 1 ml of DMEM was added to each well. Culture medium was replaced every 24 h.

Low-level laser therapy (LLLT)

After 4-day incubation, a fresh serum-free culture medium was added to the samples, and the 3D cell cultures were irradiated by means of a laser diode device (LaserTABLE, 780 nm, 0.025 W) [13–15, 25]. Energy densities of 0.5, 1.5, and 3 J/cm^2 were used to irradiate cells for three consecutive times with 24-h intervals. All irradiation parameters are shown in Table 1.

Twenty-four hours after the last irradiation, cells were subjected to evaluation of viability by alamarBlue assay and gene expression of epidermal growth factor (hEGF) and collagen type I (hCOL-I) by real-time PCR.

For this specific protocol, 3D cell cultures established as reported for the experimental groups, but not subjected to laser irradiation, were used as controls.

Histological evaluation

Histological analysis was performed for evaluation of the morphology and distribution of gingival fibroblasts cultured in the collagen matrix. For this purpose, 3D cell cultures subjected or not to laser irradiation were fixed in 10 % formaldehyde for 30 min; then, the matrices with cells were removed from the wells and transferred to plastic cassettes coated with filter paper, which were immersed again in fixative solution until analysis. Subsequently, all samples were processed with xylol, dehydrated, and then subjected to paraffin embedding. Serial sections (5 μm thick) obtained by means of a rotating microtome (RM2125RT, Leica Microsystems, Wetzlar, Germany) were stained with hematoxylin and eosin [26, 27] and analyzed under an optical microscope (Olympus BX51, Olympus America Inc., Center Valley, PA, USA). Repre-

Table 1 Irradiation parameters

Device information	
Number of emitters	12 laser diodes
Emitter type	InGaAsP laser diodes
Irradiation parameters	
Center wavelength	780 nm ± 5 nm
Operating mode	Continuous wave
Peak radiant power	70 mW
Beam profile	Gaussian
Treatment parameters	
Beam spot size at target	2 cm^2
Irradiance at target	25 mW/cm^2
Exposure duration	40 s, 120 s, 240 s
Radiant exposure	0.5 J/cm^2, 1.5 J/cm^2, 3 J/cm^2
Area irradiated	2 cm^2
Number and frequency of sessions	Three irradiations (every 24 h)

Device parameters presented according to Lins et al. (2013) [25]

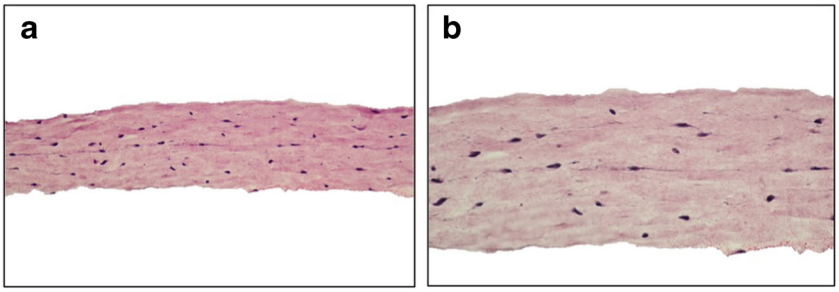

Fig. 1 Histological evaluation, control group. Photomicrographs of the nonirradiated 3D cell culture model with gingival fibroblasts seeded in type I collagen matrix. **a** H/E, 100×. **b** 200×

sentative microphotographs of experimental and control groups were obtained. For this protocol, nonirradiated 3D cell cultures were used as the control group.

Cell viability

Cell viability was assessed by alamarBlue assay (Invitrogen, Carlsbad, CA, USA) as previously described [28]. Briefly, culture medium was aspirated, and a solution of fresh serum-free culture medium containing 10 % of alamarBlue solution was added to cells and incubated for 4 h at 37 °C and 5 % of CO_2. Then, two aliquots of this solution were transferred to wells of 96-well plates, and cell viability was determined by the intensity of fluorescence (Synergy – H1, Biotek, Winooski, VT, USA; 530-nm excitation/590-nm emission).

Gene expression

Gene expression of hEGF and hCOL-I was assessed by real-time PCR. Total RNA was isolated by means of an RNAqueous micro-kit (Ambion, Grand Island, NY, USA). After LLLT, 3D cell culture was treated with lysis solution, and RNA was isolated by subsequent washing and filtration. For each sample, cDNA was synthesized by means of a High Capacity Reverse Transcriptase cDNA Kit (Applied Biosystems, Grand Island, NY, USA).

Real-time PCR reactions were performed with Taqman Master Mix and Taqman Assays at Step One Plus. Reactions were analyzed by Step One Software and normalized by expression of endogenous control (β-actin).

Statistical analysis

Numerical data of cell viability were statistically analyzed by ANOVA and Tukey tests, while qPCR data were subjected to Kruskal-Wallis complemented by Mann-Whitney tests, at the 5 % level of significance.

Results

Microphotographs of 3D cell culture demonstrated that gingival fibroblasts exhibited uniform distribution and morphology

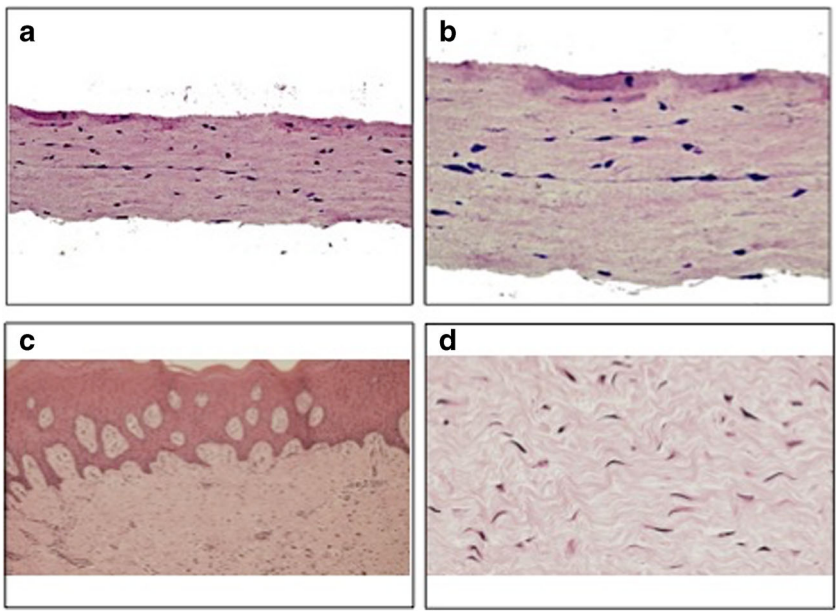

Fig. 2 Histological evaluation, experimental group. Photomicrographs of the 3D cell culture model in which gingival fibroblasts seeded in type I collagen matrix were subjected to LLLT (0.5 J/cm^2). **a** H/E, 100×. **b** 200×. **c**, **d** Histological section of gingiva obtained from a young patient. As observed in the control (nonirradiated) and experimental (irradiated) groups, the fibroblasts are uniformly distributed within the collagen matrix. **c** H/E, 100×. **d** 200×

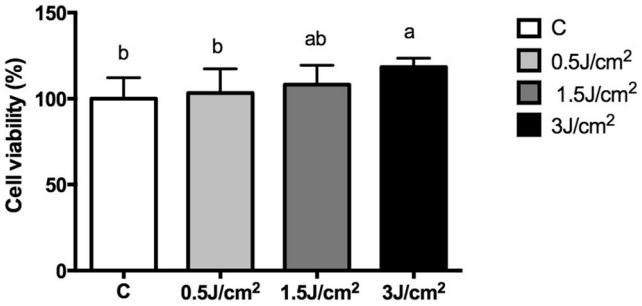

Fig. 3 Viability of gingival fibroblasts cultured in a 3D cell culture model and subjected or not (control, *C*) to laser irradiations. *Columns indicated by the same letters* do not show a statistically significant difference (ANOVA, $p > 0.05$)

within the collagen type I matrix, such as observed in the control group (Fig. 1a, b). 3D cell cultures subjected to irradiation also demonstrated uniform cell distribution and morphology [Fig. 2a, b (0.5 J/cm^2)], similar to those observed in sections obtained from biopsies of human gingiva (Fig. 2c, d).

It was determined that fibroblasts remained viable during the experiment and that the LLLT did not significantly influence their viability 24 h after the final irradiation (Fig. 3).

The evaluation of the gene expression of hEGF and hCOL-I 24 h after the final irradiation demonstrated that these genes were significantly increased when cells were irradiated at 0.5 J/cm^2. However, the other energy densities assessed in this study caused gene expression of hEGF and hCOL-I similar to that in the control group (Fig. 4a, b).

Discussion

The standardization of a 3D cell culture model for phototherapy studies is critical for the elucidation of mechanisms involved in light interactions with tissues and cells. For this reason, a 3D cell culture model with gingival fibroblasts seeded in a collagen matrix, which was subjected or not to laser irradiations, was assessed in the present investigation.

It was previously reported that extracellular matrix of gingival connective tissue is composed mainly of collagen type I [29]. Therefore, it seemed interesting to use this protein to establish a 3D model for the in vitro assessment of specific phototherapies. In the present study, the nonirradiated 3D cell culture model exhibited homogeneous distribution of gingival fibroblasts within the collagen type I matrix. This cell distribution and morphology were similar to those observed in the LLLT experimental groups as well as in histological sections of biopsies of gingivae obtained from young patients (Figs. 1 and 2). Therefore, the use of this protein in this in vitro study to produce a 3D scaffold for gingival fibroblasts can be considered as adequate to mimic the microenvironment commonly observed for in vivo situations.

In phototherapy studies with a monolayer cell culture model, the light is delivered directly to all the cells that are attached to a flat surface. However, during the clinical irradiation of damaged complex tissues, such as in oral mucositis, light has penetrated the tissue further to biostimulate the cells present in deep areas. Conversely, in this in vivo condition, the light may be absorbed by local proteins and other chromophores or even reflected by spread [11]; these events can interfere with the biostimulatory effects caused by the phototherapy [11, 19]. In the present investigation, evaluation of 3D cell culture models 24 h after the final irradiation demonstrated that the LLLT at 3 J/cm^2 enhanced gingival fibroblast viability, which may indicate an increased proliferation of the irradiated cells within the collagen matrix. All other energy densities tested did not affect cell viability. Previous studies demonstrated that LLLT with low-power laser densities (0.5 and 1.5 J/cm^2) enhanced the metabolism of gingival fibroblasts and other cell types [12–14]. However, in these studies, the laser irradiations were delivered to cells cultured in a monolayer. Therefore, it appears that higher doses of laser irradiation, such as 3 J/cm^2, are more effective for increasing the viability of fibroblasts present in deep areas of connective tissues. Thus, analysis of the data obtained in the present study indicates that the use of 3D cell culture models is adequate, and this protocol may be useful to simulate the penetration of laser light through wounded areas where connective tissue is directly exposed and cells are deeply distributed in different layers within extracellular matrix.

In the present study, evaluation of the gene expression of hEGF and hCOL-I at the time cut of 24 h after the final

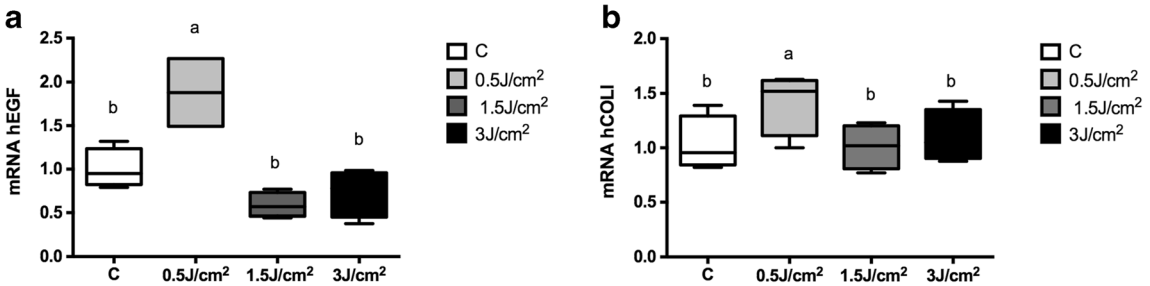

Fig. 4 Gene expression of COL-I (*a*) and EGF (*b*) by gingival fibroblasts seeded and maintained in a 3D cell culture model and subjected or not (control, *C*) to laser irradiations. *Boxplots indicated by the same letters* do not show statistically significant difference (Mann-Whitney, $p > 0.05$)

irradiation was significantly increased by LLLT at 0.5 J/cm^2, whereas for monolayer cell cultures, the expression of growth factors and collagen type I was not significantly affected by this therapy [13]. This lack of biostimulation may be caused by a possible inhibition of some phenotypic functions of cells cultured in the monolayer model [16, 30]. However, for 3D cell culture models, fibroblasts exhibit three-dimensional morphology, presenting intercellular interactions among them and also with extracellular matrix [23]. According to previous studies, this three-dimensional morphology and distribution allow cells to express surface proteins similar to those observed on in vivo tissue and show more realistic interactions with different drugs, materials, and other treatment modalities [31–33].

The hEGF gene expression was not affected by cells subjected to LLLT at 1.5 and 3 J/cm^2. This result may be related to the late gene expression assessment performed in this study. Since higher cell viability was observed for cells irradiated with 3 J/cm^2, one may suggest that higher doses of low-level laser delivered to cells cultured in a 3D model cause earlier stimulation of hEGF gene expression, which was not detected 24 h after the final irradiation. Such a hypothesis was evaluated by Hawkins-Evans and Abrahamse (2008) [34], who demonstrated that bFGF gene expression by fibroblasts was increased at 1 h after LLLT and was decreased after 24 h.

Growth factors play a fundamental role in oral mucosal healing, and the synthesis of these proteins is related to cell proliferation and migration [35, 36]. Increased EGF expression by gingival fibroblasts irradiated with a laser at 0.5 J/cm^2 indicates that a stimulatory effect may be obtained on epithelial cells by paracrine signaling. Besides stimulating keratinocyte proliferation, it is known that EGF also upregulates proliferation of gingival fibroblasts [37]. Therefore, the enhanced EGF expression observed in the 3D cell culture model irradiated with a laser at 0.5 J/cm^2 may result in the further stimulus of cell viability and proliferation.

Based on the fact that collagen is the main component of the gingival extracellular matrix [29], it seems evident that intense synthesis of collagen type I takes place during oral mucosal wound healing. After an injury, the expression and synthesis of this protein are crucial for injury closure and tissue remodeling [30]. Therefore, the increased expression of COL-I observed in the present investigation indicates that specific parameters of LLLT could be considered as interesting alternatives to biostimulate the synthesis of extracellular matrix in damaged tissues, improving local healing.

According to the methodology used in this study and based on the scientific data obtained, one may conclude that LLLT can penetrate the collagen matrix and stimulate the gingival fibroblasts seeded within it. The 3D cell culture model assessed in this in vitro investigation seems to characterize a reliable and promising laboratory method for further phototherapy studies.

Acknowledgments The authors acknowledge the Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP (grant: 2013/05879-0 and PD: 2012/17947-8) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (303599/2014 – Pq 1A and 307696/2014 Pq 1B) for financial support.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

1. Bjordal JM, Couppé C, Chow RT, Tunér J, Ljunggren EA (2003) A systematic review of low level laser therapy with location-specific doses for pain from chronic joint disorders. Aust J Physiother 49: 107–116
2. Cotler HB, Chow RT, Hamblin MR, Carroll J (2015) The use of low level laser therapy (LLLT) for musculoskeletal pain. MOJ Orthop Rheumatol 2:00068
3. Tchanque-Fossuo CN, Ho D, Dahle SE, Koo E, Li CS, Rivkah Isseroff R, Jaqdeo J (2016) A systematic review of low-level light therapy for treatment of diabetic foot ulcer. Wound Repair Regen. doi:10.1111/wrr.12399
4. Vale FA, Moreira MS, de Almeida FC, Ramalho KM (2015) Low-level laser therapy in the treatment of recurrent aphthous ulcers: a systematic review. Sci World J 2015:150412. doi:10.1155/2015/150412
5. Bjordal JM, Bensadoun RJ, Tuner J, Frigo L, Gjerde K, Lopes-Martins RA (2011) A systematic review with meta-analysis of the effect of low-level laser therapy (LLLT) in cancer therapy-induced oral mucositis. Support Care Cancer 19:1069–1077
6. Loncar B, Stipetic MM, Baricevic M, Risovic D (2011) The effect of low-level laser therapy on salivary glands in patients with xerostomia. Photomed Laser Surg 29:171–175
7. Bello-Silva MS, de Freitas PM, Aranha AC, Lage-Marques JL, Simões A, de Paula Eduardo C (2010) Low and hogh-intensity lasers in the treatment of herpes simplex virus 1 infection. Photomed Laser Surg 28:135–139
8. Muñoz Sanchez PJ, Capote Fermenías JL, Díaz Tejeda A, Tunér J (2012) The effect of 670-nm low laser therapy on herpes simplex type 1. Photomed Laser Surg 30:37–40
9. Migliorati C, Hewson I, Lalla RV, Antunes HS, Estilo CL, Hodgson B, Lopes NNF, Schubert MM, Bowen J, Elad S (2013) Systematic review of laser and other light therapy for the management of oral mucositis in cancer patients. Support Care Cancer 21:333–341
10. Ankri R, Lubart R, Taitelbaum H (2010) Estimation of the optimal wavelengths for laser-induced wound healing. Lasers Surg Med 42: 760–764
11. Frigo L, Fávero GM, Lima HJC, Maria DA, Bjordal JM, Joensen J, Iversen VV, Marcos RL, Parizzoto NA, Lopes-Martins RAB (2010) Low-level laser irradiation (InGaAlP - 660 nm) increases fibroblast cell proliferation and reduces cell death in a dose-dependent manner. Photomed Laser Surg 28:S151–S156
12. Basso FG, Pansani TN, Turrioni APS, Bagnato VS, Hebling J, Souza Costa CA (2012) In vitro wound healing improvement by low-level laser therapy application in cultured gingival fibroblasts. Int J Dent. doi:10.1155/2012/719452
13. Basso FG, Oliveira CF, Kurachi C, Hebling J, de Souza Costa CA (2013) Biostimulatory effect of low-level laser therapy on keratinocytes in vitro. Lasers Med Sci 28:367–374

14. Pansani TN, Basso FG, Turrioni APS, Kurachi C, Hebling J, de Souza Costa CA (2014) Effects of low-level laser therapy on the proliferation and apoptosis of gingival fibroblasts treated with zoledronic acid. Int J Oral Maxillofac Surg 43:1030–1034
15. Basso FG, Pansani TN, Soares DG, Scheffel DL, Bagnato VS, de Souza Costa CA, Hebling J (2015) Biomodulation of inflammatory cytokines related to oral mucositis by low-level laser therapy. Photochem Photobiol 91:952–956
16. Pampaloni F, Reynaud EG, Stelzer EH (2007) The third dimension bridges the gap between cell culture and live tissue. Nat Rev Mol Cell Biol 8:839–845
17. Edmondson R, Broglie JJ, Adcock AF, Yang L (2014) Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. Assay Drug Technol 12:207–218
18. Schwarz F, Aoki A, Sculean A, Becker J (2009) The impact of laser application on periodontal and peri-implant wound healing. Periodontol 2000 51:79–108
19. Ottaviani G, Gobbo M, Sturnega M, Martinelli V, Mano M, Zanconati F, Bussani R, Perinetti G, Long CS, De Lenarda R, Giacca M, Biasotto M, Zacchigna S (2013) Effect of class IV laser therapy on chemotherapy-induced oral mucositis. Am J Pathol 183: 1747–1757
20. Feinberg SE, Aghaloo TL, Cunningham LL (2005) Role of tissue engineering in oral and maxillofacial reconstruction: findings of the 2005 AAOMS research summit. J Oral Maxillofac Surg 63:1418–1425
21. Moharamzadeh K, Brook IM, Van Noort R, Scutt AM, Thornhill MH (2007) Tissue-engineered oral mucosa: a review of the scientific literature. J Dent Res 86:115–124
22. Liu J, Bian Z, Kuijpers-Jagtman AM, Von den Hoff JW (2010) Skin and oral mucosa equivalents: construction and performance. Orthod Craniofac Res 13:11–20
23. Moharamzadeh K, Colley H, Murdoch C, Hearnden V, Chai WL, Brook IM, Thornhill MH, MacNeil S (2012) Tissue-engineered oral mucosa. J Dent Res 91:642–650
24. Dongari-Bagtzoglou A, Kashleva H (2006) Development of a highly reproducible three-dimensional organotypic model of the oral mucosa. Nat Protoc 1:2012–2018
25. Lins EC, Oliveira CF, Guimarães OC, Costa CA, Kurachi C, Bagnato VS (2013) A novel-785nm laser diode-based system for standardization of cell culture irradiation. Photomed Laser Surg 31: 466–473
26. Izumi K, Takacs G, Terashi H, Feinberg SE (1999) Ex vivo development of a composite human oral mucosal equivalent. J Oral Maxillofac Surg 57:571–577
27. Izumi K, Song J, Feinberg SE (2004) Development of a tissue-engineered human oral mucosa: from the bench to the bed side. Cells Tissues Organs 176:134–152
28. Scheffel DLS, Soares DG, Basso FG, de Souza Costa CA, Pashley P, Hebling J (2015) Transdentinal cytotoxicity of glutaraldehyde on odontoblast-like cells. J Dent 43:997–1006
29. Bartold PM, Walsh LJ, Narayanan AS (2000) Molecular and cell biology of gingiva. Periodontol 2000(24):28–55
30. Mueller-Klieser W (1997) Three dimensional cell cultures: from molecular mechanisms to clinical applications. Am J Physiol 273: 1109–1123
31. Astashkina A, Grainger DW (2014) Critical analysis of 3-D organoid in vitro cell culture for high-throughput drug candidate toxicity assessments. Adv Drug Deliv Rev 69–70:1–18
32. Matsusaki M, Case CP, Akashi M (2014) Three-dimensional cell culture technique and pathophysiology. Adv Drug Deliv Rev 74: 95–103
33. Fitzgerald KA, Malhotra M, Curtin CM, O'Brien FJ, O'Driscoll CM (2015) Life in 3D is never flat: 3D models to optimize drug delivery. J Control Release 215:39–54
34. Hawkins-Evans D, Arahamse H (2008) Efficacy of three different laser wavelengths for in vitro wound healing. Photodermatol Photoimmun Photomed 24:199–210
35. Barrientos S, Stokadinovic O, Golinko MS, Brm H, Tomic-Canic M (2008) Growth factors and cytokines in wound healing. Wound Repair Regen 16:585–601
36. Velnar T, Bailey T, Smrkolj V (2009) The wound healing process: an overview of the cellular and molecular mechanisms. J Int Med Res 37:1528–1542
37. Werner S, Krieg T, Smola H (2007) Keratinocyte-fibroblast interactions in wound healing. J Investig Dermatol 127:998–1008