UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

LUCIANA LOURENÇO RIBEIRO VITOR

**Fatores de crescimento e síntese de proteínas na resposta celular
após aplicação do Laser de Baixa Intensidade**

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LUCIANA LOURENÇO RIBEIRO VITOR

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após aplicação do Laser de Baixa Intensidade**

Tese apresentada a Faculdade de Odontologia de Bauru da Universidade de São Paulo para obtenção do título de Doutor em Ciências no Programa de Ciências Odontológicas Aplicadas, na área de concentração Odontopediatria.

Orientadora: Profa. Dra. Thais Marchini de Oliveira

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*“O mundo não está interessado nas
tempestades que você encontrou.
Querem saber se trouxe o navio.”*

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RESUMO

Esta tese teve como objetivo verificar a resposta celular de fibroblastos pulpares após a variação nos parâmetros de fotobiomodulação. Fibroblastos pulpares de dentes decíduos humanos em 4ª passagem foram plaqueados, deixados a aderir, submetidos a privação nutricional, e em seguida irradiados com laser de baixa intensidade a 660 nm. Os grupos em estudo foram baseados na variação da potência e tempo, resultando em dosimetrias diferentes, variando entre 2,5 a 6,2 J/cm². No artigo 1, o efeito dos diferentes parâmetros da fotobiomodulação foram verificados por meio da expressão gênica do COL1 por RT-PCR, nos períodos de 6, 12 e 24 horas. No artigo 2, avaliou-se a síntese proteica de fatores angiogênicos (VEGF-A, VEGF-C, VEGF-D, VEGFR1, VEGFR2, FGF-2, PDGF, PLGF, PECAM-1, e BMP-9) por ELISA Multiplex, nos períodos de 6, 12 e 24 horas após fotobiomodulação, no sobrenadante e lisado celular. Os dados foram analisados por ANOVA a dois critérios seguido pelo teste de Tukey ($p < 0.05$) nos dois artigos. No artigo 1, a comparação intragrupo da expressão gênica do COL1 mostrou que a variação da fotobiomodulação com mais tempo (50 s) e menor potência (5 mW) em uma densidade de energia de 6,2 J/cm² mudou o padrão de expressão gênica do COL1 pelos fibroblastos pulpares de 12 h para 6 h. A comparação intergrupo da expressão genica COL1 não mostrou diferenças estatisticamente significativas. No artigo 2, fibroblastos pulpares secretaram e produziram todas as proteínas testadas antes e após a fotobiomodulação, exceto PDGF no lisado. A comparação intragrupo, no artigo 2, mostrou padrões similares da síntese proteica dos fatores angiogênicos para todos os grupos: a secreção de VEGF-A, VEGF-C, VEGFR1 e BMP-9 aumentou significativamente no sobrenadante enquanto que FGF-2 e VEGF-A aumentou significativamente no lisado. A comparação intergrupo da síntese de proteínas revelou que a dosimetria 2,5 J/cm² aumentou a secreção de VEGF-D ($p=0.4779$), VEGFR1 ($p=0.8570$) e BMP-9 ($p=0.0042$) no sobrenadante e VEGFR1 ($p=0.0128$) e VEGF-D ($p=0.0036$) no lisado; e diminuiu VEGF-A ($p=0.0176$), VEGF-C ($p=0.0328$), PDGF ($p=0.0077$), PLGF ($p=0.0004$) no sobrenadante e PLGF ($p=0.0094$) e BMP-9 ($p=0.5645$) no lisado. A dosimetria de 3.7 J/cm² aumentou VEGF-A ($p=0.8410$), FGF-2 ($p=0.4778$), BMP-9 ($p=0.0042$) e VEGFR1 ($p=0.8570$) no sobrenadante e VEGF-A ($p=0.8412$), VEGF-C ($p=0.5908$) e VEGFR1 ($p=0.0128$) no lisado; e diminuiu VEGF-A ($p=0.0176$), PDGF ($p=0.0077$) e PLGF ($p=0.0004$) no sobrenadante e PLGF ($p=0.0094$) e BMP-9 ($p=0.0276$) no lisado. Concluiu-se que as dosimetrias de 2,5 a 6,2 J/cm² biomodularam a expressão gênica de COL1, e as dosimetrias de 2,5 J/cm² e 3.7 J/cm² biomodularam a síntese proteica de vários fatores angiogênicos. Na dosimetria de 6,2 J/cm², o maior tempo e a menor potência mudaram o padrão da expressão gênica de COL1 pelos fibroblastos pulpares. A dosimetria de 3.7 J/cm² foi a mais efetiva na produção e secreção de fatores angiogênicos.

Palavras-chave: Polpa Dentária. Dente Decíduo. Terapia a Laser de Baixa Intensidade

ABSTRACT

Growth factors and protein synthesis in cellular response after photobiomodulation therapy

This thesis aimed to verify the cellular response of pulp fibroblasts after the variation in photobiomodulation parameters. Pulp fibroblasts at 4th passage were plated, led to adhere, subjected to serum starvation, and subsequently irradiated with 660 nm low-level laser. The study groups were based on the variation of the power and time, resulting in different dosimetries ranging from 2.5 to 6.2 J/cm². In article #1, the effect of different photobiomodulation parameters were verified through the COL1 gene expression by RT-PCR, at 6, 12, and 24 hours. In article #2, the protein synthesis of angiogenic factors (VEGF-A, VEGF-C, VEGF-D, VEGFR1, VEGFR2, FGF-2, PDGF, PLGF, PECAM-1, and BMP-9) was measured by ELISA Multiplex assay, at 6, 12, and 24 hours, in the supernatant and lysate. Data were analyzed by two-way ANOVA followed by Tukey test ($p < 0.05$) in both articles #1 and #2. In article #1, the intragroup comparison of the COL1 gene expression showed that the variation of PBM application with longer time (50 s) and smaller power (5 mW) at the energy density of 6.2 J/cm² changed the COL1 mRNA expression pattern by pulp fibroblasts from 12 h to 6 h. The intergroup comparison of COL1 gene expression revealed no statistically significant differences. In article #2, pulp fibroblasts secreted and produced all the tested proteins before and after PBM, except for PDGF in the lysate. The intragroup comparison, in article #2, showed similar patterns of angiogenic factors synthesis for all groups: the secretion of VEGF-A, VEGF-C, VEGFR1, and BMP-9 increased significantly in the supernatant, while FGF-2 and VEGF-A increased significantly in the lysate. The intergroup comparison of the protein synthesis revealed that the dosimetry of 2.5 J/cm² exhibited upregulation of VEGF-D ($p = 0.4779$), VEGFR1 ($p = 0.8570$), and BMP-9 ($p = 0.0042$) in supernatant and VEGFR1 ($p = 0.0128$) and VEGF-D ($p = 0.0036$) in lysate; and downregulation of VEGF-A ($p = 0.0176$), VEGF-C ($p = 0.0328$), PDGF ($p = 0.0077$), and PLGF ($p = 0.0004$) in supernatant and PLGF ($p = 0.0094$) and BMP-9 ($p = 0.5645$) in lysate. The dosimetry of 3.7 J/cm² upregulated VEGF-A ($p = 0.8410$), FGF-2 ($p = 0.4778$), BMP-9 ($p = 0.0042$), and VEGFR1 ($p = 0.8570$) in supernatant and VEGF-A ($p = 0.8412$), VEGF-C ($p = 0.5908$), and VEGFR1 ($p = 0.0128$) in lysate; and downregulated VEGF-A ($p = 0.0176$), PDGF ($p = 0.0077$), PLGF ($p = 0.0004$) in supernatant and PLGF ($p = 0.0094$) and BMP-9 ($p = 0.0276$) in lysate. We concluded that the energy densities from 2.5 to 6.2 J/cm² biomodulated the COL1 gene expression, but red laser at 2.5 J/cm² and 3.7 J/cm² biomodulated the synthesis of several angiogenic proteins. At the energy density of 6.2 J/cm², longer application time and smaller power changed the pattern of COL1 gene expression by pulp fibroblasts. However, the dosimetry of 3.7 J/cm² was the most effective for the production and secretion of angiogenic factors.

Keywords: Dental pulp. Tooth, deciduous. Low-level Light Therapy.

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1 Introduction

1 INTRODUCTION

Vital pulp therapy (VPT) is the treatment of choice for deep caries lesion with pulp vitality, comprising: selective caries removal followed by indirect pulp capping, direct pulp capping, or pulpotomy (Aapd, 2017b;a; Dhar *et al.*, 2017). VPT primarily aims to preserve the pulp capable of regenerating (Smail-Faugeron *et al.*, 2016; Aapd, 2017a;b; Coll *et al.*, 2017). Although selective caries removal avoids pulpal exposure in teeth with deep caries lesion (Aapd, 2017b; Schwendicke, 2017), sometimes pulpal exposure due to caries occurs. According to the American Association of Pediatric Dentistry's guideline, pulpotomy is the vital pulp therapy of choice for teeth with pulp exposure due to caries (Aapd, 2017b;a).

The most recent systematic review on pulp treatment for extensive decay states that Mineral Trioxide Aggregate (MTA) is the most effective medicament to heal the root pulp after pulpotomy, but MTA is expensive and further studies are necessary to find second choices (Smail-Faugeron *et al.*, 2018). In the searching for alternatives, photobiomodulation therapy (PBM) emerges for clinical use on root pulp after pulpotomy of primary teeth with successful outcomes (Fernandes *et al.*, 2015; Marques *et al.*, 2015; Uloopi *et al.*, 2016; Kuo *et al.*, 2017; Sivadas *et al.*, 2017).

PBM is the application of 600-1000 nm wavelength non-ionizing visible infrared light with output power of 1-500 mW, continuous or pulsatile radiation, in a constant beam of relatively low energy density (0.04-50 J/cm²) to inhibit or stimulate directly biological and cellular processes (Alghamdi *et al.*, 2012; Hadis *et al.*, 2016). PBM is a nonthermal process, involving endogenous chromophores, which leads to photophysical and photochemical events, such as wound healing and tissue regeneration (Anders *et al.*, 2015). These events do not follow the Law of Reciprocity, that is, the effects are not directly proportional to the total energy amount provided (Hadis *et al.*, 2016).

Accordingly, PBM depends on wavelength, dose, and application time (Karu, 1989), so that the variation in power and time results in different dosimetries and may lead to different biological responses. Dosimetry (dose, or radiant exposure, or energy density) is the amount of light leaving the laser device tip that reaches a surface (Hadis *et al.*, 2016).

To calculate the energy density directed at a spot during the therapy, first the energy (J) has to be known: $E \text{ (J)} = P \text{ (W)} \times t \text{ (s)}$, where P is the power and t the

time during the laser is used. For example, 5 mW (0.005 W) of power for 10 seconds delivers 50mW (=0.05J) of energy. The energy density is calculated as (Laakso *et al.*, 1993): $D \text{ (J/cm}^2\text{)} = E \text{ (J)}/\text{output area (cm}^2\text{)}$. If the output area is 0.04 cm², and the applied energy (E) is 0.05J, then the dose (D) is 1.2 J/cm².

PBM is dose dependent because a therapeutic window occurs between energy densities from 0.5 to 4 J/cm² (Laakso *et al.*, 1993). Moreover, the variation of power and time results in similar energy densities but the inhibitory and stimulatory effects are not fully understood on different cellular types (Hadis *et al.*, 2016).

Two mechanisms, namely primary and secondary, explain the PBM effect on the cell (Karu, 1989;1999; Karu *et al.*, 2004; Alghamdi *et al.*, 2012). The primary mechanism comprises the photochemical and photophysical events in the mitochondria (Karu, 1989; Alghamdi *et al.*, 2012) through absorption of the light by the respiratory chain components (cytochrome c oxidase) leading to the change in the mitochondrial redox state (Karu, 1989). The secondary mechanisms may occur hours or even days after the irradiation (Karu, 1999). One of this effects is on DNA because the changes in cytochrome c oxidase and redox state cause a change in biochemical reactions through photo-signal transduction and amplification chain resulting in increased proliferation, marked by DNA synthesis (Karu, 1999).

To date, the literature lacks consensus on the best combination of PBM power and time to achieve the best dose for pulp repair. Pulp repair relies on the extracellular matrix (ECM) and angiogenesis processes (Tziafas, 1995; Tziafas *et al.*, 2000; Tziafas *et al.*, 2001; Tziafas, 2004). Pulp fibroblasts play a key role in regulating pulp repair because they secrete COL1 to produce loose and dense ECM (Leblond, 1989; Kuo *et al.*, 1992) and angiogenic growth factors (Jeanneau *et al.*, 2017).

Collagens are ECM molecules used in structural integrity, tissue scaffolding, and tissue repair (Lamande and Bateman, 1999; Kadler *et al.*, 2007; Gordon and Hahn, 2010). The primary ECM component of many tissues is Collagen type 1 (COL1), including the pulp (Frantz *et al.*, 2010). On one hand, light emitting diodes (LED) and PBM modulates the collagen production in human osteoblast (Oliveira *et al.*, 2016). On the other hand, the literature lacks information on the effects of PBM on COL1 production by pulp fibroblasts.

Angiogenesis is the main process to form blood vessels, mediated by angiogenic molecules (Folkman and Shing, 1992; Yamazaki and Morita, 2006).

VEGF family proteins (VEGF-A, VEGF-C, VEGF-D, and PLGF) and receptors (VEGFR1 and VEGFR2) regulates the biological activity response of the endothelial cells through proliferation, migration, vascular permeability, and angiogenesis (Holmes *et al.*, 2007; Stüttfeld and Ballmer-Hofer, 2009; Smith *et al.*, 2015a). Other angiogenic growth factors are FGF-2, BMP-9, PDGF, and PECAM-1. FGF-2 and BMP-9 regulates angiogenesis (David *et al.*, 2008; Simons *et al.*, 2016). PDGF has an indirect angiogenic effect by maintaining and stabilizing newly-formed blood vessels (Zhang *et al.*, 2017). The literature reports the PECAM-1 regulation of angiogenesis (Lourenco-Neto *et al.*, 2018; Marques *et al.*, 2018).

Pulp fibroblasts produces angiogenic molecules and receptors for physiological angiogenesis in a paracrine manner, promoting endothelial cell chemotaxis, proliferation, and differentiation (Matsushita *et al.*, 2000; Tran-Hung *et al.*, 2006; Anca *et al.*, 2013; Chmielewsky *et al.*, 2014; Smith *et al.*, 2015b). Works from other fields show that PBM modulated the synthesis of angiogenic growth factors by mesenchymal stem cells from adipose tissue (Oliveira *et al.*, 2015) and gingival fibroblasts (Saygun *et al.*, 2008; Damante *et al.*, 2009). Recently, the literature reports that PBM at 660 nm increase VEGF gene expression in fibroblasts (Szezerbaty *et al.*, 2018), but little is known whether higher gene expression results in higher protein production and secretion.

From a more biological and less invasive point of view, the use of PBM would help in pulp repair. Considering the importance of the pulp fibroblasts in dentin-pulp regeneration after VPT and the lack of understanding about the PBM effects of different irradiances on these cells, this study aimed to verify the cellular response of pulp fibroblasts after photobiomodulation through the COL1 gene expression and protein synthesis of angiogenic factors.

2 Articles

2 ARTICLES

The articles of this thesis were written according to the submission guidelines of the corresponding journals.

- **ARTICLE 1** - Photobiomodulation changes type 1 collagen gene expression by pulp fibroblasts. Laser Physics
- **ARTICLE 2** - Photobiomodulation upregulates angiogenic protein synthesis by pulp fibroblasts. Plos One

2.1 Article #1 - Photobiomodulation changes type 1 collagen gene expression by pulp fibroblasts

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Photobiomodulation changes type 1 collagen gene expression by pulp fibroblasts

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Abstract

This study aimed to evaluate type 1 collagen (COL1) gene expression by human pulp fibroblasts from primary teeth (HPF) after the variation of photobiomodulation (PBM) parameters. HPF were obtained from a biorepository, used at 4th passage, and irradiated (InGaAlP—660 nm) varying the power and application time according to the following groups: G1: 1.2 J cm⁻²–05 mW–10 s; G2: 2.5 J cm⁻²–05 mW–20 s; G3: 3.7 J cm⁻²–05 mW–30 s; G4: 5.0 J cm⁻²–05 mW–40 s; G5: 6.2 J cm⁻²–05 mW–50 s; G6: 2.5 J cm⁻²–10 mW–10 s; G7: 3.7 J cm⁻²–15 mW–10 s; G8: 5.0 J cm⁻²–20 mW–10 s; G9: 6.2 J cm⁻²–25 mW–10 s. The control group (G10) was not irradiated and maintained with DMEM + 10% SFB. RT-PCR was used to evaluate COL1 gene expression at 6, 12, and 24 h after irradiation. Intra- and intergroup comparisons were performed by two-way ANOVA followed by Tukey test ($p < 0.05$). The intragroup comparison showed statistically significant differences among periods ($p < 0.05$). In G1, G9, and G10 higher COL1 gene expression occurred at 12 h ($p = 0.07$), while in G2 to G8, the peak of higher expression occurred at 6 h ($p = 0.02$). The intergroup comparison did not show statistically significant differences ($p > 0.05$). The energy densities from 2.5 to 5 J cm⁻², regardless of the variation in PBM parameters, biomodulated the COL1 gene expression. At the energy density of 6.2 J cm⁻², longer application time and smaller power changed the pattern of COL1 gene expression by pulp fibroblasts from human primary teeth.

Keywords: photobiomodulation, collagen, deciduous tooth, vital pulp therapy

(Some figures may appear in colour only in the online journal)

2.2 Article #2 - Photobiomodulation upregulates angiogenic protein synthesis by pulp fibroblasts

ABSTRACT

This study aimed to assess the protein synthesis of angiogenic factors by pulp fibroblasts from human teeth (HPF) after photobiomodulation (PBM). HPF at 4th passage was plated, led to adhere, subjected to serum starvation, and subsequently irradiated with 660 nm low-level laser according to the following groups: 2.5 J/cm² - 10 mW/10 sec ; 3.7 J/cm² - 15 mW/10 sec. The control group was not irradiated. The synthesis of the following angiogenic factors was obtained by ELISA Multiplex Assay: VEGF-A, VEGF-C, VEGF-D, VEGFR1, VEGFR2, FGF-2, PDGF, PLGF, PECAM-1, and BMP-9 at 6, 12, and 24 hours after PBM, in supernatant and lysate. Two-way ANOVA followed by Tukey test were applied to test intra- and intergroup comparisons ($P < 0.05$). HPF secreted and produced all the tested proteins before and after PBM, except for PDGF in the lysate. The intragroup comparisons showed similar patterns of angiogenic factors synthesis for all groups: the secretion of VEGF-A, VEGF-C, VEGFR1, and BMP-9 increased significantly in the supernatant, while FGF-2, VEGF-A increased significantly in the lysate ($p < 0.05$). The dosimetry of 2.5 J/cm² exhibited upregulation of VEGF-D ($p = 0.4779$), VEGFR1 ($p = 0.8570$), and BMP-9 ($p = 0.0042$) in supernatant and VEGFR1 ($p = 0.0128$) and VEGF-D ($p = 0.0036$) in lysate; and downregulation of VEGF-A ($p = 0.0176$), VEGF-C ($p = 0.0328$), PDGF ($p = 0.0077$), PLGF ($p = 0.0004$) in supernatant and PLGF ($p = 0.0094$), BMP-9 ($p = 0.5645$) in lysate. The dosimetry of 3.7 J/cm² had upregulation of VEGF-A ($p = 0.8410$), FGF-2 ($p = 0.4778$), BMP-9 ($p = 0.0042$), and VEGFR1 ($p = 0.8570$) in supernatant and VEGF-A ($p = 0.8412$), VEGF-C ($p = 0.5908$), and VEGFR1 ($p = 0.0128$) in lysate; and downregulation of VEGF-A ($p = 0.0176$), PDGF ($p = 0.0077$), PLGF ($p = 0.0004$) in supernatant and PLGF ($p = 0.0094$), and BMP-9 ($p = 0.0276$) in lysate. The dosimetries of 2.5 J/cm² and 3.7 J/cm² biomodulates the synthesis of several angiogenic proteins. However, the dosimetry of 3.7 J/cm² was the most effective for the production and secretion of angiogenic factors

Keywords: Photobiomodulation. Angiogenesis Factor. Deciduous tooth. Vital pulp therapy.

1 - Introduction

Angiogenesis is the main process to form blood vessels in wound repair mediated by angiogenic molecules [1, 2]. VEGF family proteins (VEGF-A, VEGF-C, VEGF-D, and PLGF) and receptors (VEGFR1 and VEGFR2) regulate the biological activity response of the endothelial cells through proliferation, migration, vascular permeability, and angiogenesis [3-5]. Other angiogenic growth factors are FGF-2, BMP-9, PDGF, and PECAM-1. FGF-2 and BMP-9 regulates angiogenesis [6, 7]. PDGF has an indirect angiogenic effect by maintaining and stabilizing newly-formed blood vessels [8]. The literature reports the PECAM-1 regulation of angiogenesis [9, 10].

One of the pulp regeneration keystones is angiogenesis [11, 12]. Pulp fibroblasts produces angiogenic molecules and receptors for physiological angiogenesis in a paracrine manner, promoting endothelial cell chemotaxis, proliferation, and differentiation [12-16]. Different stress conditions, such as caries or trauma, result in different composition and concentration of the secretome from pulp cells [17].

Works from other fields reveal that photobiomodulation (PBM) modulated the synthesis of angiogenic growth factors by mesenchymal stem cells from adipose tissue [18] and gingival fibroblasts [19, 20]. Recently, the literature reports that PBM at 660 nm increase VEGF gene expression in fibroblasts [21], but little is known whether higher gene expression results in higher protein production and secretion. Clinically, PBM has been successfully used in vital pulp therapy [22, 23], but the literature lacks a protocol regarding the most effective PBM parameters. PBM biological effects depend on wavelength, dose, and application time [24], so that different dosimetries may result in different biological responses [25].

Therapies that keeps pulp vitality after caries or trauma relies on the pulp tissue capable of regenerating [26, 27]. From a more biological and less invasive point of view, the use of PBM would help in pulp repair. Thus, this study aimed to assess the protein synthesis of angiogenic factors by pulp fibroblasts after photobiomodulation.

2 - Material and methods

2.1 - Cell culture

Pulp fibroblasts from human primary teeth (HPF) were obtained through a Biorepository [28, 29]. 2×10^4 cells/well were seeded in 24-well plate (code 3524, polystyrene, Corning, NY, USA) with Dulbecco's modified Eagle's medium (DMEM - Sigma Chemical Co, St. Louis, MO) supplemented with 10% Fetal Bovine Serum (FBS), incubated at 37°C and 5% CO₂, for 24 hours to adhere. The culture medium was replaced by DMEM supplemented with 1% FBS to synchronize the cell cycle prior to irradiation and achieve proliferative deficit [28, 30]. After 24 h, prior to irradiation, the culture medium was replaced by DMEM without phenol red (Sigma Chemical Co, St. Louis, MO) supplemented with 10% FBS for all groups [28]. Groups were plated in biological triplicate (3 wells per group).

2.2 PBM irradiation

The study groups were divided according to different dosimetries: 2.5 J/cm² - 10 mW/10 sec; 3.7 J/cm² - 15 mW/10 sec. The control group was not irradiated (control). Aluminum-gallium-indium-phosphide (InGaAlP) diode laser (Twin Flex Evolution MMOptics® – São Carlos, Brazil), at 660nm wavelength, 0.04 cm² laser tip area, 0.225 cm tip diameter, continuous mode was applied according to the study groups. Based on previous studies [28, 30] a black mask covers the culture plate, the distance between the laser beam and the cell culture was standardized at 1 mm, and the output power was checked by a radiometer (Laser Check MMOptics® – São Carlos/SP, BR), prior to laser application. The control group (not irradiated) was maintained in DMEM+10%FBS [28, 30].

2.3 Collection of supernatant and lysate

After 6, 12 and 24 hours, the supernatant of all groups was collected and stored for the analysis. After the collection of the supernatant, 300 ul/well of lysis solution (CellyticnM, Sigma Aldrich, C2978, 50MI) was dispensed to obtain the chemical lysis. Also, the mechanical lysis was performed by scratching the pipette tip. The lysate of all groups was collected and stored for analysis.

2.4 Immunoassay procedure

The following panels were used to verify the protein synthesis of VEGF-A, VEGF-C, VEGF-D, VEGFR1, VEGFR2, FGF2, PLGF, PDGF, PECAM-1, and BMP-9 at 6, 12 and 24 hours after irradiation, in supernatant and lysate: HAGP1MAG-12K

(MILLIPLEX® MAP Human Angiogenesis Panel 1) and HANG2MAG-12K (MILLIPLEX® MAP Human Angiogenesis Panel 2). All samples, quality control samples and standards were prepared as recommended in the MILLIPLEX® map assay kit protocols with supplied diluents, following the manufacturer's instructions. The assay plate was then analyzed with the Luminex® instrument equipped with xPONENT® and Multiplex Analyst 5.1 software. The results were obtained in pg/ml.

2.5 - Statistical analysis

All statistical analyses were obtained with level of significance of 5% (Prism Graph Pad 7 for Windows). Intra- and intergroup comparisons were analyzed by two-way ANOVA followed by Tukey test.

3 – Results

3.1 - Intragroup comparison

In control group, HPF secreted all the tested proteins before PBM, except for PDGF in the lysate. Non-irradiated HPF secreted higher levels of VEGF-A, VEGF-C, and VEGFR2, but low levels of FGF-2, PDGF, VEGFR1, PECAM-1, VEGF-D, PLGF, and BMP-9 in supernatant (Table 1). In lysate, non-irradiated HPF produced higher levels of FGF-2, VEGF-C, and VEGFR2, but lower levels of VEGFR1, VEGF-A, PECAM-1, VEGF-D, PLGF, BMP-9 (Table 1). Over time, the secretion of VEGF-A, VEGF-C, VEGFR1, and BMP-9 by non-irradiated HPF increased significantly in the supernatant, while FGF-2, VEGF-A increased significantly in the lysate ($p < 0.05$). In the supernatant of non-irradiated HPF, VEGFR2 and PLGF decreased the synthesis overtime, while FGF-2 had a peak of maximum secretion at 12 h, and PDGF, PECAM-1, VEGF-D, and BMP-9 had different secretion patterns, but without statistically significant differences ($p > 0.05$). In the lysate of non-irradiated HPF, VEGFR2, VEGF-C, VEGFR1 decreased the synthesis over time, while PECAM1, VEGF-D, PLGF, BMP-9 was constant, but without statistically significant differences ($p > 0.05$).

Table 1 – Angiogenic protein synthesis by non-irradiated HPF (control group)

SUPERNATANT				
PROTEINS	6h	12h	24h	P value
VEGF-C	66.48±1.75 ^a	115.22±5.00 ^b	201.46±34.23 ^c	<0.0001*
VEGF-A	31.03±4.00 ^a	69.00±9.96 ^b	214.70±23.50 ^c	<0.0001*
VEGFR2	31.33±2.22 ^a	30.05±0.00 ^a	30.05±0.00 ^a	0.7230
FGF-2	17.66±5.01 ^a	36.63±42.89 ^a	22.36±3.14 ^a	0.6113
PDGF	7.65±2.84 ^a	8.33±1.24 ^a	10.88±4.06 ^a	0.7879
VEGFR1	6.00±0.48 ^a	7.35±0.80 ^a	17.13±2.42 ^b	<0.0001*
PECAM-1	3.67±0.25 ^a	3.38±0.00 ^a	3.52±0.25 ^a	0.3153
VEGF-D	2.24±0.25 ^a	2.39±0.00 ^a	2.47±0.14 ^a	0.5768
PLGF	1.04±0.13 ^a	1.01±0.09 ^a	0.98±0.13 ^a	0.2807
BMP-9	0.61±0.10 ^a	0.72±0.04 ^{ab}	0.76±0.02 ^b	0.0140*
LYSATE				
PROTEINS	6h	12h	24h	P value
FGF-2	1296.67±233.14 ^a	1420.33±179.51 ^{ab}	1754.33±311.59 ^b	0.0005*
VEGFR2	30.58±2.16 ^a	29.33±0.00 ^a	28.12±2.10 ^a	0.6734
VEGF-C	26.06±1.56 ^a	22.06±3.07 ^a	23.99±2.71 ^a	0.0515
VEGFR1	10.74±0.00 ^a	7.12±3.24 ^a	8.39±6.01 ^a	0.1741
VEGF-A	8.78±0.36 ^a	10.07±0.39 ^a	14.46±0.39 ^b	<0.0001*
PECAM-1	3.24±0.24 ^a	3.52±0.43 ^a	3.24±0.24 ^a	0.7874
VEGF-D	2.38±0.24 ^a	2.52±0.00 ^a	2.52±0.00 ^a	0.4728
PLGF	0.60±0.05 ^a	0.55±0.00 ^a	0.55±0.00 ^a	0.6410
BMP-9	0.52±0.00 ^a	0.52±0.00 ^a	0.49±0.05 ^a	0.5645
PDGF	0.00	0.00	0.00	-----

* Different superscript lowercase letters in row mean statistically significant differences among periods (Two-way ANOVA followed by Tukey test; P<0.05). Values in pg/ml.

HPF irradiated with 2.5 J/cm² secreted all tested proteins, except for PDGF in the lysate. The quantification of the proteins in both supernatant and lysate after irradiation with 2.5 J/cm² showed a pattern similar to that of control group. Overtime, VEGF-A, VEGF-C, and VEGFR1 increased significantly in supernatant (p<0.05), while FGF-2, VEGF-A, and VEGF-D increased significantly in the lysate (p<0.05) (Table 2). The other angiogenic proteins had different patterns of synthesis overtime, but without statistically significant differences (p>0.05) (Table 2).

Table 2 – Angiogenic protein synthesis by HPF irradiated with 2.5 J/cm².

SUPERNATANT				
PROTEINS	6h	12h	24h	P value
VEGF-C	62.88±4.60 ^a	115.87±4.54 ^b	154.59±49.39 ^b	<0.0001*
VEGF-A	29.59±3.07 ^a	60.48±8.28 ^b	142.41±18.00 ^c	<0.0001*
VEGFR2	30.05±0 ^a	31.33±2.22 ^a	31.33±2.22 ^a	0.7230
FGF-2	15.99±8.70 ^a	16.25±5.98 ^a	12.52±1.13 ^a	0.6113
VEGFR1	5.68±0.27 ^a	8.43±0.84 ^a	16.90±3.91 ^b	<0.0001*
PDGF	5.30±0.74 ^a	3.70±2.90 ^a	5.63±1.73 ^a	0.7879
PECAM-1	3.38±0 ^a	3.38±0 ^a	3.52±0.25 ^a	0.3153
VEGF-D	2.39±0 ^a	2.55±0.28 ^a	2.24±0.25 ^a	0.5768
PLGF	0.52±0.04 ^a	0.52±0.04 ^a	0.54±0.00 ^a	0.2807
BMP-9	0.77±0 ^a	0.72±0.04 ^a	0.72±0.04 ^a	0.5190
LYSATE				
PROTEINS	6h	12h	24h	P value
FGF-2	1240.67±85.17 ^a	1545±156.51 ^{ab}	1818.67±78.42 ^b	0.0005*
VEGFR2	29.36±3.69 ^a	28.12±2.10 ^a	30.58±2.16 ^a	0.6734
VEGF-C	24.89±1.12 ^a	25.07±2.14 ^a	25.52±2.34 ^a	0.0515
VEGFR1	17.60±4.13 ^a	21.21±9.94 ^a	11.37±3.57 ^a	0.1741
VEGF-A	9.00±0.74 ^a	10.58±2.46 ^{ab}	13.45±1.98 ^b	<0.0001*
PECAM-1	3.52±0 ^a	3.38±0.24 ^a	3.38±0.24 ^a	0.7874
VEGF-D	2.38±0.24 ^a	2.52±0 ^a	2.95±0.00 ^b	0.0123*
PLGF	0.47±0.08 ^a	0.47±0 ^a	0.50±0.05 ^a	0.6410
BMP-9	0.47±0.05 ^a	0.47±0.05 ^a	0.47±0.05 ^a	0.5645
PDGF	-----	----	----	-----

* Different superscript lowercase letters in row mean statistically significant differences among periods (Two-way ANOVA followed by Tukey test; P<0.05). Values in pg/ml.

HPF irradiated with 3.7 J/cm² secreted all tested proteins, except for PDGF in the lysate. The quantification of the proteins in both supernatant and lysate after irradiation with 3.7 J/cm² showed a pattern similar to that of control group (Table 3). Overtime, VEGF-A, VEGF-C, and VEGFR1 increased significantly in supernatant (p<0.05), while FGF-2 and VEGF-A, increased significantly in the lysate after irradiation with 3.7 J/cm² (p<0.05) (Table 3). The other proteins had different patterns of synthesis overtime, but without statistically significant differences (p>0.05) (Table 3).

Table 3 – Angiogenic protein synthesis by HPF irradiated wit 3.7 J/cm².

SUPERNATANT				
PROTEINS	6h	12h	24h	P value
VEGF-C	62.81±8.54 ^a	115,86±4.17 ^b	207.85±10.98 ^c	<0.0001*
VEGF-A	37.03±2.75 ^a	60,60±9.73 ^a	178.75±19.02 ^b	<0.0001*
VEGFR2	31.33±2.22 ^a	30,05±0 ^a	30.05±0 ^a	0.7230
FGF-2	21.56±7.86 ^a	16,89±5.68 ^a	16.06±3.89 ^a	0.6113
PDGF	6.41±1.80 ^a	7,00±1.45 ^a	4.78±1.77 ^a	0.7879
VEGFR1	4.94±0.66 ^a	8,53±0.98 ^b	18.50±0.73 ^c	<0.0001*
PECAM-1	3.67±0.25 ^a	3,52±0.25 ^a	///3.38±0 ^a	0.3153
VEGF-D	2.24±0.25 ^a	2,24±0.25 ^a	2.39±0 ^a	0.5768
BMP-9	0.76±0.06 ^a	0,75±0.04 ^a	0.75±0.04 ^a	0.5190
PLGF	0.66±0.05 ^a	0,61±0 ^a	0.69±0.08 ^a	0.2807
LYSATE				
PROTEINS	6h	12h	24h	P value
FGF-2	1253.67±234 ^a	1435,00±244,01 ^{AB}	1732.67±178.35 ^b	0.0005*
VEGFR2	29.33±0 ^a	29.33±0 ^a	29.33±0 ^a	0.6734
VEGF-C	28.05±0.96 ^a	23,27±3.52 ^a	24.39±2.53 ^a	0.0515
VEGF-A	10.30±0.78 ^a	10.31±1.40 ^a	13.91±2.91 ^b	<0.0001*
VEGFR1	6.11±0 ^a	13.75±7.57 ^a	9.14±5.25 ^a	0.1741
PECAM-1	3.24±0.24 ^a	3.38±0.24 ^a	3.52±0 ^a	0.7874
VEGF-D	2.52±0 ^a	2.66±0.25 ^a	2.52±0 ^a	0.4409
PLGF	0.47±0.08 ^a	0.50±0.05 ^a	0.52±0.05 ^a	0.6410
BMP-9	0.47±0.05 ^a	0.44±0 ^a	0.44±0 ^a	0.5645
PDGF	-----	-----	----	----

* Different superscript lowercase letters in row mean statistically significant differences among periods (Two-way ANOVA followed by Tukey test; P<0.05). Values in pg/ml.

3.1 - Intergroup comparison

The results were expressed as percentage of controls, using the values from Table 1.

In supernatant, at 6 hours, the dosimetry of 3.7 J/cm² non-significantly increased the secretion of VEGF-A (p=0.8410) and FGF-2 (p=0.4778) (Figure 1A and 1E). Both dosimetries significantly biomodulated the BMP-9 secretion (p=0.0042), after 6 hours in supernatant (Figure 1F). At 12 hours, both dosimetries increased VEGFR1 secretion in supernatant without statistically significant differences (p=0.8570) (Figure 1D). The dosimetry of 2.5 J/cm² increased the VEGF-D secretion in supernatant without statistically significant differences (p=0.4779), at 12 hours (Figure 1C). At 24 hours, VEGF-A secretion in the supernatant significantly decreased to lower values after PBM with both dosimetries (p=0.0176) (Figure 1A). At this period, VEGF-C secretion significantly decrease after PBM application with 2.5 J/cm² (p=0.0328) (Figure 1B). The dosimetry of 3.7 J/cm² had significant higher synthesis of VEGF-A (p=0.0083) and VEGF-C (p=0.0150) than the dosimetry of 2.5 Jcm² in supernatant at 24h (Figure 1A and 1B). Both dosimetries downregulated the PDGF secretion in supernatant, at all periods, with statistically significant differences (p=0.0077) at 24 hours (Figure 1I). Statistically significant lower values of PLGF secretion in the supernatant occurred at all periods (p=0.0004) for both dosimetries (Figure 1J). At all periods, VEGFR2 (Figure 1E) and PECAM-1 (Figure 1H) had values statistically similar to that of control group for both dosimetries.

Figure 1A-J – Intercomparison of the quantification of angiogenic factors after PBM in supernatant
 * indicates a significant difference with the control considering each angiogenic factor and each period separately (Two-way ANOVA; $P < 0.05$). # indicates a significant difference between the dosimetries of 2.5 and 3.7 J/cm² considering each angiogenic factor and each period separately (Two-way ANOVA; $P < 0.05$)

In lysate, at 6 hours, the dosimetry of 3.7 J/cm² non-significantly increased the production of VEGF-A (p=0.8412) and VEGF-C (p=0.5908) (Figure 2A and 2B). The dosimetry of 2.5 J/cm² biomodulated the VEGFR1 production in the lysate at 6 hours without statistically differences with control group (p=0.2871), but with significant differences with the dosimetry of 3.7 J/cm² (p=0.0440) (Figure 2D). Statistically significant lower values of PLGF production (p=0.0094) in lysate occurred at 6 hours for both dosimetries (Figure 2I). After 12 hours, both dosimetries increased the VEGFR1 production in lysate, with statistically significant differences with control group for 2.5 J/cm² (p=0.0128) (Figure 2D). Both dosimetries decreased the BMP-9 production in the lysate, with statistically significant difference (p=0.0276) for 3.7 J/cm², at 12 hours (Figure 2F). At 24 hours, VEGF-D production in the lysate significantly increased after PBM with 2.5 J/cm² compared with control group and the dosimetry of 3.7 J/cm² (p=0.0036) (Figure 2C). At all periods, VEGFR2 (Figure 2E) and PECAM-1 (Figure 2H) had values statistically similar to that of control group for both dosimetries. At all periods, PDGF was not produced in the lysate for both dosimetries and control group.

Figure 2A-I – Intergroup comparison of the quantification of angiogenic factors after PBM in lysate

* indicates a significant difference with the control considering each angiogenic factor and each period separately (Two-way ANOVA; $P < 0.05$). # indicates a significant difference between the dosimetries of 2.5 and 3.7 J/cm² considering each angiogenic factor and each period separately (Two-way ANOVA; $P < 0.05$)

4 - Discussion

This study showed the photobiomodulation of the main angiogenic factors in supernatant and lysate. As far as we are concerned, this is the first study addressing the synthesis of angiogenic proteins by pulp fibroblasts after PBM, which makes comparisons difficult.

In terms of biomodulation, the higher power and dose upregulated the angiogenic factors (VEGF-A, VEGF-C, FGF-2, BMP-9, and VEGFR1 in supernatant and VEGF-A and VEGF-C in lysate). In general, photobiomodulation of angiogenesis increases VEGF production due to nitric oxide modulation [21, 31]. This occurs through nitric oxide production synthase via activation of PLC γ -Ca dependent influx [32-34].

FGF-2 plays a key role in pulp repair by regulating angiogenesis [35]. Although through different methodologies and with different cell lines, in general, PBM upregulates FGF-2 synthesis [19, 20, 36, 37], similarly to the result of this study. Moreover, FGF-2 synergistically potentializes the action of VEGF on endothelial cells, which in turn increases VEGF receptor expression [6, 13].

The higher dose (3.7 J/cm²) also upregulated the VEGFR1 synthesis. VEGFR1 controls vascular biology acting as “VEGF trap” by sequestering VEGF-A away from VEGFR2 and forming non-signaling VEGFR1-VEGFR2 heterodimer through dimerization, transphosphorylation, and regulation of expression levels [6, 15, 34, 38, 39].

In supernatant, 24 hours after PBM, the secretion of VEGF-A, VEGF-C, and FGF2 was statistically lower than control group for both doses, but with markedly decreasing for the lower dose (2.5 J/cm²). The regulation of VEGF family proteins and receptors is a complex interplay [40]. Many factors regulated VEGF synthesis: VEGFR1 (as mentioned above) [6, 15, 34, 38, 39], VEGF bioavailability through either plasmin cleavage or extracellular proteolysis [3, 6], splicing of VEGF proteins affecting temporal and spatial distribution [34], FGF controlling of VEGF upstream [6], and VEGFR2 binding to integrin [6, 33, 34, 38]. Integrin is a transmembrane heterodimer that mediates cell-matrix adhesion through binding to extracellular proteins, such as collagen, fibronectin, and laminin [38]. Integrin recruitment qualitatively modulates the signaling of VEGF receptors [34, 38] and the VEGFR2 binding to integrin prevents aberrant angiogenesis stimulation [6, 33]. PBM increases

COL1 gene expression by HPF [30]. Collagen 1 downregulated the VEGF-A mediated activation of VEGFR2 through phosphatase SHP2 recruitment [38]. Taken together, these information suggest the crosstalk between proteins of the signaling cascade of VEGF protein and receptors.

After PBM, BMP-9 was upregulated in supernatant and downregulated in lysate with both dosimetries. In dentistry, the literature reported an increase in BMP-9 levels after PBM with different methodologies (immunohistochemistry) and cells (osteoblasts) [41]. Upon BMP-9 stimulation, stem cells reveal odontoblastic differentiation [42]. Further studies are necessary to understand the role of secreted BMP-9, after PBM, in pulp angiogenesis and repair.

Both dosimetries downregulated the synthesis of PDGF (supernatant) and PLGF (supernatant and lysate). To the best of our knowledge, the literature lacks studies on the protein synthesis and production of PLGF by pulp fibroblasts. Studies on angiogenesis show that PLGF plays a crucial role in inflammation-associated angiogenesis by recruiting inflammatory cells, mediated by binding VEGFR1 [6, 34, 39]. The literature reported an increase in PDGF levels after PBM, but with different wavelength (1064 nm) and dosimetry (1.6 J/cm²) on different cells (palatal tissue) [41, 43]. Works from other fields highlighted the inhibitory effect of PBM in PDGF-stimulated cells [44]. Although Rombouts, Giraud (17) reports the increase of PDGF transcription by pulp cells, we did not find the same protein transduction in this study.

The methodology of this study was based on previous research [28-30]. We used 660 nm wavelength based on the maintenance of HPF viability and proliferation [28] and effect in gene expression [18, 21, 30]. Before PBM, HPF secreted and produced all the tested angiogenic factors, except for PDGF in lysate, which agrees with the literature [13, 45-49]. Other studies show that to simulate pulp stress conditions either hypoxia or serum deprivation lead to different angiogenic secretion by pulp cells [17, 50, 51]. This would explain the synthesis and production of angiogenic proteins by non-irradiated HPF in this present study. Further studies are necessary to verify whether hypoxia would change the angiogenic secretome of pulp fibroblasts [52]

5 - Conclusion

The dosimetries of 2.5 J/cm² and 3.7 J/cm² biomodulates the synthesis of several angiogenic protein. However, the dosimetry of 3.7 J/cm² was the most effective for the production and secretion of angiogenic factors.

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3 Discussion

3 DISCUSSION

This section provides the discussion of important ideas of methodology, results, and clinical relevance of the study.

3.1 Methodology

The methodology of the two studies were based on previous research (Lourenço-Neto *et al.*, 2017; Marques *et al.*, 2017; Fernandes *et al.*, 2018). We used 660-nm wavelength based on the maintenance of HPF viability and proliferation (Marques *et al.*, 2017; Fernandes *et al.*, 2018), effect in gene expression (Oliveira *et al.*, 2015; Szezerbaty *et al.*, 2018) and on the assumption that the visible spectrum would have effects on protein synthesis (Alghamdi *et al.*, 2012). The used irradiances ranged from 1.2 to 6.2 J/cm², that is, irradiances shorter than those reported to decrease immortalized oral fibroblast viability (Engel *et al.*, 2016).

Recently, a study found that the optical parameters, biological factors, protocols, and oxygen level in cell culture medium impacts the results of the experiment in dermal fibroblasts (Mignon *et al.*, 2017). Other studies show that to simulate pulp stress conditions either hypoxia or serum deprivation lead to different angiogenic secretion by pulp cells (Aranha *et al.*, 2010; Bakopoulou *et al.*, 2015; Rombouts *et al.*, 2017). This would explain the synthesis and production of angiogenic proteins by non-irradiated HPF in this present study. Further studies are necessary to verify whether hypoxia would change the angiogenic secretome of pulp fibroblasts (Mignon *et al.*, 2017)

3.2 Results

Photobiomodulation upregulates the COL1 gene expression and the synthesis of angiogenic factors by pulp fibroblasts. Considering the two articles together, the energy density of 3.7 J/cm² (15 mW/10 sec) was the most effective considering COL1 gene expression and protein synthesis of angiogenic factors.

While some studies found similar results of increased COL1 gene expression by irradiated fibroblasts (Martignago *et al.*, 2015; Pansani *et al.*, 2017), others concluded that the amount of COL1 expression was not different between irradiated and non-irradiated fibroblasts (Pereira *et al.*, 2002; Marques *et al.*, 2004). The

rationale behind this distinct outcome is the different cell type (gingival fibroblasts rather than pulp fibroblasts), wavelength (904 nm), irradiance (3 J/cm^2), and application time. Different cell types have dissimilar morphological patterns leading to distinct secretion of ECM components (Martinez and Araujo, 2004). Thus, different PBM parameters would result in different biological responses (Huang et al., 2009), and would affect collagen gene expression (Zhang et al., 2003).

Considering the synthesis of angiogenic factors, the literature reports that photobiomodulation increases VEGF production due to nitric oxide modulation (Prindeze et al., 2012; Szezerbaty et al., 2018), through nitric oxide production synthase via activation of PLC γ -Ca dependent influx (Cébe-Suarez et al., 2006; Koch et al., 2011; Fraisl, 2013). PBM also upregulates FGF-2 synthesis (Saygun et al., 2008; Damante et al., 2009; Aykol et al., 2011; Oliveira et al., 2017). Moreover, FGF-2 synergistically potentializes the action of VEGF on endothelial cells, which in turn increases VEGF receptor expression (Tran-Hung et al., 2006; Simons et al., 2016). In dentistry, the literature reported an increase in BMP-9 levels after PBM, similar to the results of the Article #2, but with different methodologies (immunohistochemistry) and cells (osteoblasts) (Tim et al., 2014). The literature reported an increase in PDGF levels after PBM, but with different wavelength (1064 nm) and dosimetry (1.6 J/cm^2) on different cells (palatal tissue) (Tim et al., 2014; Keskiner et al., 2016), which was different from the result of Article #2.

We suggest a cross-talking in the signaling cascade of VEGF proteins and receptors because the secretion of VEGF-A, VEGF-C, and FGF-2 were lower than the control group, 24 hours after the irradiation with 2.5 J/cm^2 . One of the mechanisms of VEGF control is the VEGFR2 binding to integrin, a transmembrane heterodimer that mediates cell-matrix adhesion through binding to extracellular proteins, such as collagen (Cébe-Suarez et al., 2006; Koch et al., 2011; Koch and Claesson-Welsh, 2012; Simons et al., 2016). Other mechanism of VEGF control is the collagen 1 downregulation mediated by the activation of VEGFR2 through phosphatase SHP2 recruitment (Koch and Claesson-Welsh, 2012). As seen in Article #1, PBM increases COL1 gene expression by pulp fibroblast, which could have influenced the signaling cascade of angiogenic factors.

3.3 Clinical relevance

Despite the successful clinical outcomes of PBM use in pulp vital therapy of primary teeth (Fernandes *et al.*, 2015; Marques *et al.*, 2015; Aapd, 2017b;a; Dhar *et al.*, 2017), the literature lacks a PBM protocol with the most effective wavelength, power, and application time tailored to achieve dentin-pulp regeneration in vital pulp therapy of primary teeth. Both Articles #1 and #2 provide a guideline for the clinical use of PBM, based on *in vitro* gene expression and synthesis of endogenous growth factors aiming at pulp regeneration

4 Conclusions

4 CONCLUSIONS

Conclusion Article #1 – The energy densities from 2.5 to 6.2 J/cm² biomodulated the COL1 gene expression. At the energy density of 6.2 J/cm², longer application time and smaller power changed the pattern of COL1 gene expression by pulp fibroblasts from HPF.

Conclusion Article #2 - The dosimetries of 2.5 J/cm² and 3.7 J/cm² biomodulates the synthesis of several angiogenic protein. However, the dosimetry of 3.7 J/cm² was the most effective for the production and secretion of angiogenic factors.

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Annexes

ANNEX A – Ethical approval

FACULDADE DE
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PARECER CONSUBSTANCIADO DO CEP**DADOS DO PROJETO DE PESQUISA**

Título da Pesquisa: Fatores de crescimento e síntese de proteínas na resposta celular após aplicação do Laser de Baixa Intensidade

Pesquisador: Luciana Lourenço Ribeiro Vitor

Área Temática:

Versão: 1

CAAE: 54859816.1.0000.5417

Instituição Proponente: Universidade de São Paulo

Patrocinador Principal: FUNDAÇÃO DE AMPARO À PESQUISA DO ESTADO DE SÃO PAULO

DADOS DO PARECER

Número do Parecer: 1.512.428

Apresentação do Projeto:

A polpa do dente pode ser acometida por inflamação e infecção o que pode acarretar na necessidade de sua retirada e consequentemente em tratamento endodôntico.

Polpa de dente decíduo responde basicamente da mesma forma que polpa de dente permanente frente a estes desafios, porém tratamento endodôntico em crianças, sempre que possível, deve ser evitado. Pensando nisso, o presente projeto de pesquisa se propõe a estudar a influência da ação do laser de baixa intensidade em fibroblastos de polpa de dente decíduo em sua viabilidade e expressão de genes importantes na homeostasia e na resposta a inflamação/infecção na tentativa de se evitar a pulpectomia. Os poucos relatos presentes na literatura sobre a ação de laser de baixa intensidade em células e tecidos sugerem ação antiinflamatória significativa.

Objetivo da Pesquisa:

O objetivo do presente projeto será avaliar o efeito de diferentes intensidades do Laser de Baixa Intensidade:

1. Na viabilidade de fibroblastos derivados da polpa de dentes decíduos humanos;
2. Na expressão gênica de RNAm para VEGF, FGF-2, Colágeno tipo 1 e DMP-1

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Telefone: (14)3235-8356 **Fax:** (14)3235-8356 **E-mail:** ocp@fob.usp.br

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Continuação do Parecer: 1.512.426

3. Na cinética de expressão das proteínas VEGF e FGF-2
4. Na detecção da produção proteica de Colágeno tipo 1

Avaliação dos Riscos e Benefícios:

Não há riscos na execução deste projeto uma vez que as células a serem utilizadas se encontram em cultura sem a necessidade de nova coleta de polpa de dente decíduo.

Os benefícios esperados serão compreender como reagem os fibroblastos em cultura frente a estímulos com laser de baixa intensidade. Isto poderá contribuir para a proposta de tratamento fotodinâmico em eventualidades odontológicas tais como inflamação e/ou infecção pulpar.

Comentários e Considerações sobre a Pesquisa:

Pesquisas básicas entrelaçadas com a realidade clínica são essenciais para a proposição dos tratamentos odontológicos sempre visando o bem estar do paciente. A aplicação dos objetivos do presente projeto à prática clínica não é imediata, porém norteia futuras pesquisas que poderão ser realizadas "in vivo" para melhor compreensão da ação fotodinâmica nos tecidos orais humanos.

Considerações sobre os Termos de apresentação obrigatória:

Os termos apresentados estão adequados.

Recomendações:

Não há recomendações.

Conclusões ou Pendências e Lista de Inadequações:

Julgo aprovado o presente projeto.

Considerações Finais a critério do CEP:

Esse projeto foi considerado APROVADO na reunião ordinária do CEP de 20.04.2016, com base nas normas éticas da Resolução CNS 466/12. Ao término da pesquisa o CEP-FOB/USP exige a apresentação de relatório final. Os relatórios parciais deverão estar de acordo com o cronograma e/ou parecer emitido pelo CEP. Alterações na metodologia, título, inclusão ou exclusão de autores, cronograma e quaisquer outras mudanças que sejam significativas deverão ser previamente comunicadas a este CEP sob risco de não aprovação do relatório final. Quando da apresentação deste, deverão ser incluídos todos os TCLEs e/ou termos de doação assinados e rubricados, se pertinentes.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

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ANNEX B – Ethical approval of the Biorrepository

<div style="display: flex; justify-content: space-between; align-items: center;"><div style="text-align: center;">FACULDADE DE ODONTOLOGIA DE BAURU- USP</div><div style="text-align: right;"> </div></div>
PARECER CONSUBSTANCIADO DO CEP
<p>DADOS DO PROJETO DE PESQUISA</p> <p>Título da Pesquisa: ISOLAMENTO, CULTIVO E CARACTERIZAÇÃO DE CÉLULAS DA POLPA DE DENTES DECÍDUOS</p> <p>Pesquisador: Thais Marchini de Oliveira</p> <p>Área Temática:</p> <p>Versão: 3</p> <p>CAAE: 49806215.6.0000.5417</p> <p>Instituição Proponente: Universidade de São Paulo</p> <p>Patrocinador Principal: Financiamento Próprio</p> <p>DADOS DO PARECER</p> <p>Número do Parecer: 1.389.213</p> <p>Apresentação do Projeto: Idem ao parecer 1.292.563</p> <p>Objetivo da Pesquisa: Idem ao parecer 1.292.563</p> <p>Avaliação dos Riscos e Benefícios: Idem ao parecer 1.292.563</p> <p>Comentários e Considerações sobre a Pesquisa: Idem ao parecer 1.292.563</p> <p>Considerações sobre os Termos de apresentação obrigatória: Os documentos exigidos por este CEP, foram apresentados de forma correta, efetuando as adequações sugeridas pela relatoria.</p> <p>Recomendações:</p> <p>Conclusões ou Pendências e Lista de Inadequações: A pesquisadora responsável atendeu satisfatoriamente as solicitações de ajustes nos documentos necessários, tais como: Regulamento do Biorrepositório e Termo de Consentimento Livre e Esclarecido. Desta forma sou de parecer favorável à aprovação do projeto de pesquisa em análise.</p>
<div style="font-size: small;"><p>Endereço: DOUTOR OCTAVIO PINHEIRO BRISOLLA 75 QUADRA 9</p><p>Bairro: VILA NOVA CIDADE UNIVERSITARIA CEP: 17.012-901</p><p>UF: SP Município: BAURU</p><p>Telefone: (14)3235-8356 Fax: (14)3235-8356 E-mail: cep@fob.usp.br</p></div>

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Continuação do Parecer: 1.389.213

lembrando que um relatório final deverá ser apresentado a este CEP para análise, ao final do cronograma estabelecido, salientando que quaisquer alterações que possam ocorrer, durante o desenvolvimento do trabalho de pesquisa, sejam informadas, com antecedência, para análise deste CEP.

Considerações Finais a critério do CEP:

Esse projeto foi considerado APROVADO ad referendum, nesta data, com base nas normas éticas da Resolução CNS 466/12. Ao término da pesquisa o CEP-FOB/USP exige a apresentação de relatório final. Os relatórios parciais deverão estar de acordo com o cronograma e/ou parecer emitido pelo CEP. Alterações na metodologia, título, inclusão ou exclusão de autores, cronograma e quaisquer outras mudanças que sejam significativas deverão ser previamente comunicadas a este CEP sob risco de não aprovação do relatório final. Quando da apresentação deste, deverão ser incluídos todos os TCLEs e/ou termos de doação assinados e rubricados, se pertinentes.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BASICAS_DO_PROJETO_571417.pdf	08/12/2015 13:38:17		Aceito
Outros	RegulamentoBiorrepositorioOdontopFOBUSP2015.pdf	08/12/2015 10:58:27	Thais Marchini de Oliveira	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLEReformulado2.pdf	08/12/2015 10:55:46	Thais Marchini de Oliveira	Aceito
Projeto Detalhado / Brochura Investigador	ProjetodetalhadoReformulado.pdf	29/10/2015 17:49:13	Thais Marchini de Oliveira	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLEReformulado.pdf	29/10/2015 17:48:31	Thais Marchini de Oliveira	Aceito
Outros	QuestionarioTecnicoPesquisador.pdf	08/09/2015 15:28:18	Thais Marchini de Oliveira	Aceito
Outros	DeclaracaoCompromissoPesquisadorresponsavel.pdf	08/09/2015 15:27:44	Thais Marchini de Oliveira	Aceito
Projeto Detalhado / Brochura Investigador	Projetodetalhado.pdf	08/09/2015 15:26:06	Thais Marchini de Oliveira	Aceito
Outros	Termodedoacao.pdf	18/08/2015 20:50:41	Thais Marchini de Oliveira	Aceito

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