Lasers Med Sci (2015) 30:1815–1822
DOI 10.1007/s10103-014-1656-7

ORIGINAL ARTICLE

Low-level laser therapy as an alternative for pulpotomy in human primary teeth

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Received: 12 March 2014 / Accepted: 9 September 2014 / Published online: 21 September 2014

Abstract This study aimed to evaluate the effects of low-level laser therapy (LLLT) on pulpal response of primary teeth. Twenty mandibular primary molars were randomly divided into the following groups: group I Buckley's formocresol (diluted at 1:5), group II calcium hydroxide, group III LLLT+zinc oxide/eugenol, and group IV LLLT+calcium hydroxide. LLLT parameters were set at 660-nm wavelength, 10-mW power output, and 2.5 J/cm^2 energy density for 10 s in continuous mode (InGaAlP laser, Twin Laser®, MMOptics, Sao Carlos, Sao Paulo, Brazil). The teeth were extracted at the regular exfoliation period. The dentin-pulp complex was graded by an established histopathological score system. Statistical analysis was performed by Kruskal-Wallis and chi-square test. The histopathological assessment revealed statistically significant differences among groups ($P<0.05$). The lowest degree of pulpal inflammation was present in LLLT+calcium hydroxide ($P=0.0296$). Calcium hydroxide showed the highest rate of hard tissue barrier ($P=0.0033$), odontoblastic layer ($P=0.0033$), and dense collagen fibers ($P=0.0095$). On the other hand, formocresol showed the highest incidence of internal resorption ($P=0.0142$). Based on this study, low-level laser therapy preceding the use of calcium hydroxide exhibited satisfactory results on pulp tissue healing. However, further clinical studies on human teeth with long-term follow-up are needed to test the low-level laser therapy efficacy.

Keywords Tooth deciduous · Calcium hydroxide · Laser therapy low level · Pulpotomy · Dental pulp

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Introduction

Pulpotomy is one of the most commonly used treatments for managing extensive caries in primary molars [1]. Although different capping agents for pulpotomy of primary teeth are widely studied, the literature lacks of consensus on the most appropriate therapy [2–4]. The study on different alternatives aims to find an option that presents good clinical efficacy without adverse effects.

In this context, the use of low-level laser therapy (LLLT) has shown potential in stimulating the repair and healing of dental tissue [5–9]. Furthermore, this therapy provides pain reduction and edema regression, with consequent anti-inflammatory effects [7, 10, 11]. From a biological point of view, LLLT offers low energy density for the target cell in order to stimulate membranes or organelles, leading to positive biomodulation. The change on cellular metabolism that occurs toward establishing the normality state of the affected area is known as biostimulation [10, 12].

LLLT can reduce the exudative phase of inflammatory process, accelerate epithelialization, promote higher degree of vascularization, and increase the collagen synthesis [10, 11, 13–15]. The mitochondrial respiratory chain components absorb the laser energy and converted it into metabolic energy, thus increasing adenosine triphosphate (ATP). Simultaneously, ATP regulates protein and DNA syntheses, consequently increasing expression of growth factors and cytokines, leading to cell proliferation, thus

improving regeneration [12]. This therapy has also shown increased synthesis of endorphins, decreased bradykinin, and altered pain threshold [11, 16]. Despite of the advantages presented by this minimally invasive therapy, the biological mechanism of laser-tissue interaction is not completely understood [11, 17].

The literature report few studies on pulpotomy of primary teeth using different laser types, with varied wavelengths and energy dosages, resulting in confusing evidence regarding LLLT influence on pulp tissue repair process [5–9, 15, 18, 19].

This study aimed to evaluate the effects of low-level laser therapy on pulpal response of primary teeth.

Null hypothesis

The null hypothesis for this study was that low-level laser therapy would have no effect on histological pulpal response of human primary teeth after pulpotomy.

Methods

Participants

The Ethics Committee of Bauru School of Dentistry, University of Sao Paulo, approved the protocol of this study (process no. 058/2011). At pretreatment screening period, the parents or guardians of the children received information concerning the procedures involved in the study and signed informed consent forms.

The inclusion criteria for tooth selection comprised the following: children aged between 6 and 9 years with cooperative behavior, mandibular primary first or second molar compromised by deep caries with vital pulp; absence of spontaneous pain history; no clinical or radiographic evidence of fistula or abscess, internal root resorption, inter-radicular, and/or furcal bone destruction; and the possibility of proper tooth restoration. Exclusion criteria consisted of the presence of systemic pathology and history of allergic reaction to latex, to local anesthetics, or to the constituents of the pulp dressing agents tested.

Twenty primary molars (11 first and 9 second primary molars) from 16 children (10 females and 6 males, ranging from 6 to 9 years, mean age of 8 years and 1 month,) were randomly allocated to the four treatment groups.

Clinical procedures and laser irradiation

Twenty mandibular primary molars were assigned by a random-number-producing system (computerized random numbers) into the following groups: group I (FC) Buckley's formocresol (diluted at 1:5), group II (CH) calcium hydroxide, group III (LLLT+ZOE) low-level laser therapy+zinc oxide/eugenol, and group IV (LLLT+CH) low-level laser therapy+calcium hydroxide.

Two previously calibrated pediatric dentists performed all pulpotomies. The treatment protocol was accomplished at standardized single visit as previously described by Moretti et al. [20]. After local anesthesia (4 % articaine with 1:100,000 epinephrine) and rubber dam isolation, caries was removed through round bur at low speed. The pulp chambers were opened with round bur (no. 1014, no. 1015, KG Sorensen, Cotia, SP, Brazil) at high speed, under water spray. Complete coronal pulp tissue was removed manually with an excavator, followed by irrigation with saline solution to clear off the debris. The wound surface was continuously irrigated with saline solution. A dry sterile cotton pellet was placed on the radicular pulp stumps under slight pressure for 5 min until hemostasis was achieved.

In FC group, a sterile cotton pellet dampened with diluted FC (1:5 Buckley's solution, Biodinâmica Química e Farmacêutica Ltda., Ibiporã, PR, Brazil) was placed on the amputated pulp and removed after 5 min. Next, the pulp stumps were covered with zinc oxide-eugenol paste (ZOE). In CH group, the canal orifices were dressed with calcium hydroxide (Biodinâmica Química e Farmacêutica Ltda., Ibiporã, PR, Brazil) with the aid of a sterile amalgam carrier. In LLLT+ZOE group, after hemorrhage control, laser radiation (InGaAlP laser, Twin Laser®, MMOptics, Sao Carlos, Sao Paulo, Brazil) was delivered through 320-μm-diameter optical fiber in contact with pulp tissue. LLLT parameters were set at 660-nm wavelength, 10-mW power output, 2.5 J/cm^2 energy density, 50–60-Hz frequency, 0.04-cm^2 focus beam diameter, and irradiation time of 10 s in continuous mode [9]. Then, zinc oxide-eugenol base was placed on the pulp chamber. In LLLT+CH group, all initial steps were similar to the previous group, except for the dressing material placement. After LLLT application, calcium hydroxide was placed into pulp chamber with the aid of a sterile amalgam carrier as in CH group. All groups received a layer of reinforced zinc oxide-eugenol (IRM®, Dentsply, Petrópolis, RJ, Brazil) prior to resin-modified glass ionomer cement restoration (Vitremer®, 3M ESPE, Sao Paulo, SP, Brazil). Immediate postoperative periapical radiographs were taken to assure that the dressing agents were correctly placed over the remaining radicular pulp. These radiographs were used as initial parameters for further postoperative evaluations.

Histological analysis

Periodic follow-up examinations were carried out at 3-monthly intervals after the ending of the treatment by two pediatric dentists. The pulpotomized teeth were extracted at the regular exfoliation period [21]. Following extraction, the teeth were immediately fixed in 10 % formalin for 24 h, and

then decalcified in 4 % EDTA for 45–60 days. Next, the teeth were processed for histological analysis. Longitudinal serial sections were cut at a setting of 5 μm of thickness, stained with hematoxylin and eosin, and assessed under a light microscope [4].

Mean tooth extraction time was 11.73 months. During the evaluation period, one child from LLLT+CH group gave up on participating of the study at 3-month follow-up. Two teeth from CH group had no sufficient remaining root after histological processing and were excluded. At the end of the study, 17 teeth were available to be evaluated microscopically.

Dentin-pulp complex morphological analysis was based on the histopathological evaluation described by Caicedo et al. [22], comprising the following criteria: pulp vitality, inflammation, hard tissue barrier formation, odontoblastic layer, pulp calcification, granulation tissue, internal resorption, vascularization, and collagen fibers. Two blinded and previously calibrated investigators (k=0.94 for inter-examiner reproducibility) performed the histological analysis through an established score system [22]. The parameters were individually analyzed regarding the presence (score 1) or absence (score 0), quantity, and/or concentration of the observed structures. Particularly, inflammation was scored as 0 (none), 1 (mild), 2 (moderate), 3 (intense), or 4 (necrosis), while vascularization was scored as 0 (absent), 1 (regular amount of blood vessels), or 2 (great amount of blood vessels), and collagen fibers were scored as 0 (absent), 1 (loose), or 2 (dense).

Statistical analysis

Data were submitted to statistical analysis through STATISTICA 9 software (StatSoft Inc, Tulsa, Oklahoma, USA). Inter-examiner reproducibility was determined by Kappa test. Kruskal-Wallis test followed by Dunn test was used to determine significant differences of the ordinal-qualitative variables (pulp inflammation, vascularization, and collagen fibers). Chi-squared test followed by multiple-comparison test was used to determine significant differences of nominal-qualitative variables (pulp vitality, hard tissue barrier, odontoblastic layer, pulpal calcification, granulation tissue, and internal resorption). Statistical significance was established at 5 %.

Results

All groups demonstrated clinical and radiographic success during the observed period. Histopathological analysis showed that all teeth maintained pulp vitality in the remaining radicular pulp tissues, with different degrees of inflammation. In FC group, most cases presented moderate inflammatory infiltration and regular vascularization in the loose connective tissue. A superficial necrotic layer surrounded by a band of inflammatory cells was observed in some cases. Internal resorption was a common finding adjacent to the pulp area in contact with the material. None of the cases showed hard tissue barrier, odontoblastic layer or granulation tissue. The remaining root of the two teeth presented pulp calcification (Fig. 1). CH group showed variable inflammatory infiltration according to the mononuclear cells concentration observed in the dense connective tissue. In all cases, there was formation of hard tissue barrier underlined by the odontoblastic layer and regular vascularization. Pulp calcification and internal resorption occurred in two cases. Granulation tissue and giant cells surrounding the remaining material on the hard tissue barrier was noted in one case (Fig. 2). In LLLT+ZOE group, mild to moderate inflammatory infiltration and regular vascularization in the loose connective tissue were detected in most cases. Presence of pulp calcification and absence of hard tissue barrier, odontoblastic layer, granulation tissue, and internal resorption were observed in all cases (Fig. 3). In LLLT+CH group, most cases presented dense conjunctive tissue, regular vascularization, and hard tissue barrier over the odontoblastic layer. Internal resorption and granulation tissue were not observed in this group. Two teeth showed pulp calcification in the remaining roots (Fig. 4).

The teeth were scored according to a previously established score system (Table 1) and submitted to statistical analysis. The pulpal inflammation showed statistically significant differences between FC and LLLT+CH groups, with the latter presenting the least degree. Concerning to collagen fibers, hard tissue barrier, and odontoblastic layer, differences were seen between FC and CH groups and between CH and LLLT+ZOE groups. Accordingly, CH group showed the greatest degree of dense collagen fibers, hard tissue barrier, and odontoblastic layer. Statistically significant differences were seen between FC and LLLT+ZOE groups and between FC and LLLT+CH groups for internal resorption. FC group showed the greatest internal resorption rate. There was no statistically significant difference among groups for pulpal vitality, vascularization, pulpal calcification, and granulation tissue (Table 2).

Discussion

Low-level laser therapy has been proposed as an alternative for pulpotomies of primary teeth, although the laser action on maintaining pulp vitality of human primary teeth is not completely understood [7, 9, 19]. LLLT effects on the repair process are related with the stimulation of cellular proliferation increase [11, 12, 14]. This therapy has significant effects on the synthesis, releasing, and metabolism of a range of neurotransmitters, causing significantly increasing in fibroblast production and activity, which accelerate collagen

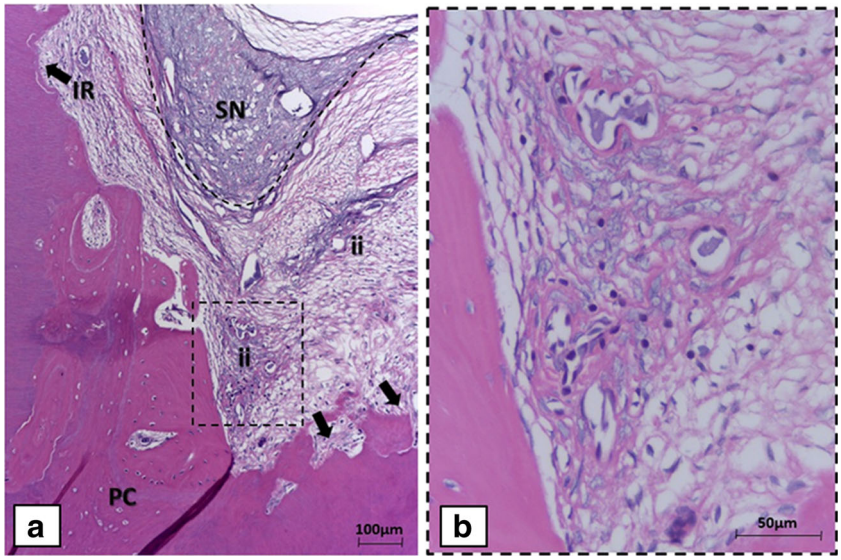

Fig. 1 Formocresol group. **a** Loose connective tissue with mild inflammatory infiltrate (*ii*), superficial necrotic layer (*SN*), internal resorption (*IR*, *arrow*), and pulp calcification (*PC*). **b** Higher magnification of the detached area in (**a**) showing the mild mononuclear inflammatory infiltrate in detail. Hematoxylin and eosin staining; scale bar of 100 (**a**) and 50 μm (**b**)

synthesis [11]. First, helium-neon lasers were the most employed, later replaced by semiconductor diode lasers (λ= 635 to 830 nm) [10, 23]. Accordingly, this study employed diode laser (active medium: InGaAlP, λ=660 nm) in LLLT+ZOE and LLLT+CH groups.

There is a paucity of information about the optimum dosage to be used on connective tissue to provide biological repair [17]. The results of a review study indicate that lasers with wavelength ranging from 600 to 700 nm (visible spectrum) and energy density from 0.5 to 4.0 J/cm^2 have shown the most effective outcomes in cellular proliferation. The stimulation of cellular proliferation is dependent on the dosage of laser irradiation. Lower doses (around 675 nm; 2 J/cm^2) increase the cell proliferation and other cellular functions, while higher doses (greater than 810 nm; 10 J/cm^2) have inhibitory effects [12, 16]. Thus, it is suggested that the dosimetry used in this study (660 nm; 2.5 J/cm^2) enhanced cellular activities and supported the pulp tissue repair process.

Fernandes et al. [9] used the same laser radiation parameters of this study to compare the clinical and radiographic effectiveness of LLLT on pulpotomy of primary molars. All the teeth treated by pulpotomy technique demonstrated 100 % of clinical success rates over the follow-up period. The radiographic success rate of teeth treated with LLLT preceding the use of calcium hydroxide ranged from 85.7 to 75 % at 6 to 18 months of follow-up. Golpayegani et al. [7] performed a randomized clinical trial that evaluated the effects of a diode laser with 632-nm wavelength on pulpotomy of primary teeth. This investigation showed, respectively, 100 and 89 % of clinical and radiographic success at 6-month follow-up. Durmus and Tamboga [19] treated 120 primary molars by pulpotomy technique with formocresol, ferric sulfate, and 810-nm diode laser. The clinical success rates at 12-month follow-up were 97, 95, and 100 %, whereas the radiographic success rates were 87, 79, and 75 % for the formocresol, ferric sulfate, and diode laser groups, respectively. The findings of this present study are in agreement with these previous

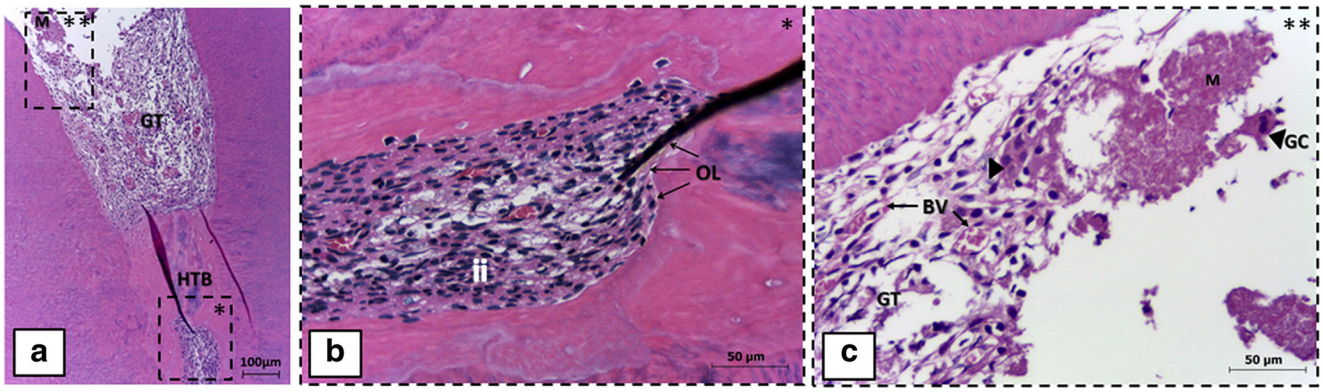

Fig. 2 Calcium Hydroxide group. **a** Hard tissue barrier (*HTB*), granulation tissue (*GT*), and remaining material (*M*). **b** Higher magnification of the detached area (*asterisk*) in (**a**) and shows odontoblastic layer (*OL*, *arrow*), and moderate mononuclear inflammatory infiltrate (*ii*); **c** Higher magnification of the detached area (*double asterisk*) in (**a**) and shows granulation tissue (*GT*) with blood vessels (*BV*), mononuclear inflammatory cells, remaining material (*M*), and giant cells (*GC solid arrow*). Hematoxylin and eosin staining; scale bar of 100 (**a**) and 50 μm (**b**, **c**)

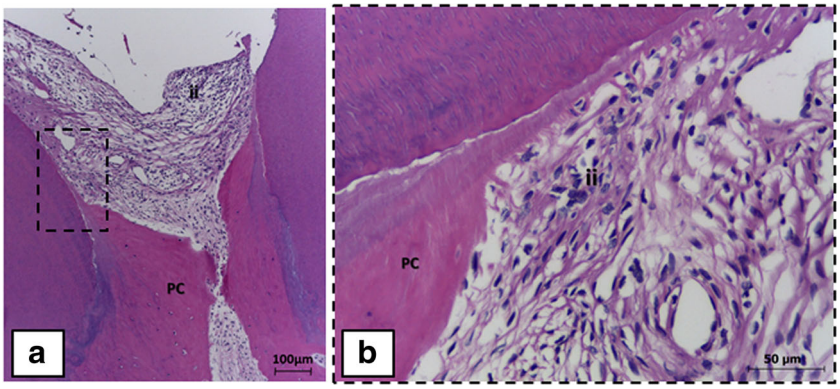

Fig. 3 LLLT+zinc oxide and eugenol group. **a** Loose connective tissue with moderate inflammatory infiltrate (*ii*) and pulp calcification (*PC*), better observed in higher magnification **b**. Hematoxylin and eosin staining; scale bar of 100 (**a**) and 50 μm (**b**)

investigations, suggesting that LLLT maintains pulp vitality and does not cause adverse effects on pulpotomy of primary teeth.

Although LLLT action is not clearly established, some studies have suggested this technology promotes biomodulation on dental pulp cell activity, biostimulation of reactional dentin, and less intense inflammatory process before the restoration procedure [24]. LLLT applied on dentin pulp interface after cavity preparation seems to accelerate the recovery of dental structure. The rationale behind this affirmation is that LLLT induces improvement in calcification on the wound surface and stimulates formation of calcified tissue [25]. Furthermore, low-level lasers have been used as a suitable approach to treat dentin hypersensitivity [26] and to reduce pain caused by dental procedures [16].

Despite of the reported high clinical and radiographic success indexes of different pulp capping materials, many studies have described pulpal inflammatory alterations when a histological evaluation was performed [4, 22, 27]. Thus, to judge pulp capping material effectiveness, it is important to determine microscopically the cellular organization, absence/presence of inflammation (type and severity), necrosis, resorption, and hard tissue barrier formation [28]. This investigation showed the LLLT histological effects as pulpotomy technique in decayed human primary teeth compared to other pulp capping materials. Scoring criteria previously described in the literature were adapted and used to assess the pulp response to different capping agents [13, 29–35].

As expected and confirmed by these results, formocresol triggered an unfavorable histological response in the remaining radicular pulp, displaying areas of necrosis and connective tissue with chronic inflammation varying from low to high [6, 27, 32, 36]. Although in the present study, no tooth showed pulp necrosis, in the research of Haghgoo and Abbasi [36], 36.3 % of the teeth treated with formocresol triggered this alteration. In some of the cases, a layer of superficial necrosis onto the inflamed area was detected. This finding was not considered as a failure, but the result of the mummification caused by formocresol action [33]. Although some studies on animals have reported hard tissue barrier formation with the use of FC [32, 33], no hard tissue barrier or odontoblastic layer were observed in FC group suggesting that formocresol does not induce tissue repair [37].

In CH group, the degree of pulp inflammation varied from absent to mild/moderate. The variation of chronic inflammatory response to calcium hydroxide has been previously described [4, 21, 27, 30, 31]. All teeth from CH group exhibited hard tissue barrier formation above the odontoblastic layer and dense connective tissue. Such findings are in agreement with other studies reporting the formation of hard tissue barrier in histological analyses of

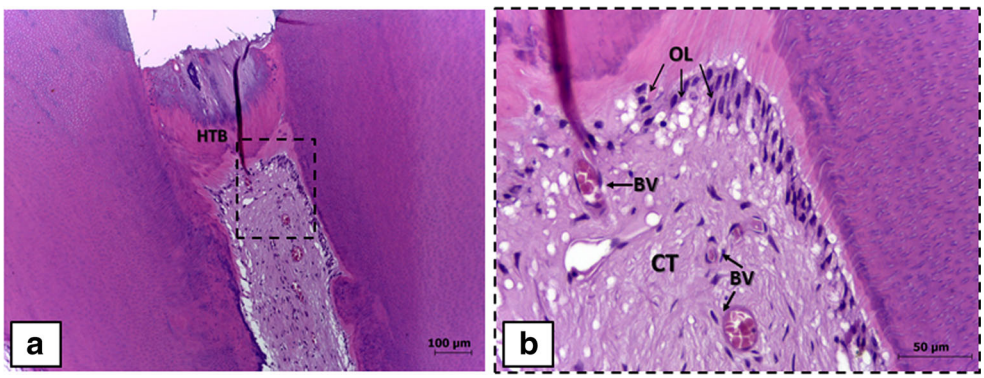

Fig. 4 LLLT+calcium hydroxide group. **a** Hard tissue barrier (*HTB*). **b** Higher magnification of the detached area in (**a**) and shows dense connective tissue (*CT*), blood vessels (*BV*), absence of inflammatory infiltrate and odontoblastic layer (*OL*). Hematoxylin and eosin staining; scale bar of 100 (**a**) and 50 μm (**b**)

Table 1 Score system used for histopathological evaluation of the pulpotomized teeth

Criteria	Scores	FC	CH	LLLT+ZOE	LLLT+CH
Pulp vitality	0	0	0	0	0
	1	5 (100 %)	3 (100 %)	5 (100 %)	4 (100 %)
Pulp inflammation	0	0	1 (33.33 %)	0	3 (75 %)
	1	1 (20 %)	1 (33.33 %)	3 (60 %)	1 (25 %)
	2	2 (40 %)	1 (33.33 %)	2 (40 %)	0
	3	2 (40 %)	0	0	0
	4	0	0	0	0
Vascularization	0	0	1 (33.33 %)	0	0
	1	5 (100 %)	2 (66.67 %)	4 (80 %)	3 (75 %)
	2	0	0	1 (20 %)	1 (25 %)
Collagen fibers	0	0	0	0	0
	1	5 (100 %)	0	5 (100 %)	2 (50 %)
	2	0	3 (100 %)	0	2 (50 %)
Hard tissue barrier	0	5 (100 %)	0	5 (100 %)	1 (25 %)
	1	0	3 (100 %)	0	3 (75 %)
Odontoblastic layer	0	5 (100 %)	0	5 (100 %)	1 (25 %)
	1	0	3 (100 %)	0	3 (75 %)
Pulp calcification	0	3 (60 %)	1 (33.33 %)	0	2 (50 %)
	1	2 (40 %)	2 (66.67 %)	5 (100 %)	2 (50 %)
Granulation tissue	0	5 (100 %)	2 (66.67 %)	5 (100 %)	4 (100 %)
	1	0	1 (33.33 %)	0	0
Internal resorption	0	1 (20 %)	1 (33.33 %)	5 (100 %)	4 (100 %)
	1	4 (80 %)	2 (66.67 %)	0	0

teeth treated with calcium hydroxide in cases of pulpal exposure [4, 21, 27, 29–31]. Either complete or partial hard tissue barrier may be presented promoting none or little communication between the capping material and the pulpal cells [31]. Interestingly, one tooth from CH group displayed the formation of connective tissue onto the hard tissue barrier, suggesting that this tissue remnant could have surpassed the incomplete barrier. In this case, giant cells were observed encompassing the material remnants. The permanence of calcium hydroxide particles associated with the presence of giant cells was also observed in other studies [29, 31].

Additionally to CH, LLLT+CH groups displayed hard tissue barrier formation and odontoblastic layer.

Table 2 Statistical analysis results of the groups using the Kruskal-Wallis test followed by Dunn test for the ordinal-qualitative variables and chi-squared test followed by a multiple comparison test for nominal-qualitative variables

	FC	CH	LLLT+ZOE	LLLT+CH	p
Pulp vitality	1A	1A	1A	1A	>0.05
Pulp inflammation	2.2A	1AB	1.4AB	0.25B	0.0296*
Vascularization	1A	0.66A	1.4A	1.25A	>0.05
Collagen fibers	0.8A	2B	1A	1.5AB	0.0095*
Hard tissue barrier	0A	1B	0A	0.75AB	0.00332*
Odontoblastic layer	0A	1B	0A	0.75AB	0.00332*
Pulp calcification	0.4A	0.66A	1A	0.5A	>0.05
Granulation tissue	0A	0.33A	0A	0A	>0.05
Internal resorption	0.8A	0.66AB	0B	0B	0.01425*

Same letters indicate no statistically significant difference among the groups (horizontal line)

*$p<0.05$, statistically significant difference

These results corroborate other studies suggesting that pulp repair can be attributed to calcium hydroxide capability of inducing and regulating the differentiation of odontoblast-like and odontoblast cells in depositing mineralized matrix [4, 29–31] LLLT+ZOE group did not show the formation of the hard tissue barrier, agreeing with the findings described by Toomarian et al. [34].

The groups treated with LLLT did not show internal resorption. FC group histologically displayed the highest degree of internal resorption. Other studies demonstrated higher indexes of inflammation and internal resorption for formocresol when compared with those of laser therapy [6, 34]. In CH group, there was a predominance of internal resorption. Although this alteration suggests pulp vitality, internal resorption results from odontoclast activity, indicating chronic inflammation of the residual pulp. However, the exact mechanism accounting for this process remains not yet properly understood. Other factors as inadequate control of hemorrhage during the procedure, contact of the material with the blood clot, improper restorations, and pulp inflammation have been related to this alteration [8, 9, 20, 21, 27].

LLLT+ZOE and LLLT+CH groups exhibited increased amounts of vessels. As previously described in the literature, LLLT has demonstrated an effective action in many biological processes, such as the following: the increase of mitochondrial respiration and ATP synthesis, modulation of inflammatory processes, acceleration of regenerative process and soft tissue healing, and angiogenesis [17].

The statistical analysis of the present study showed that the groups submitted to laser therapy presented more satisfactory responses regarding to repair process than the group undergoing formocresol. LLLT+CH group displayed the smallest degree of inflammation. Internal resorption was greater in FC group than in LLLT+ZOE and LLLT+CH groups. Considering the hard tissue barrier, odontoblast layer, and dense collagen fibers, the capping agent used in CH group demonstrated to be better than those in FC and LLLT+ZOE groups.

The limitations of the present study include the disadvantages of noncompliance and high dropout rates, and the obstacle to control the period of tooth extraction. These disadvantages result in investigations with small sample size and short follow-up time [38]. Further studies should be conducted to determine optimum and safe laser parameters, and the results should be confirmed through longer-term following-up periods.

Conclusions

Based on this study, low-level laser therapy preceding the use of calcium hydroxide exhibited satisfactory results on pulp tissue healing. However, further clinical studies on human teeth with long-term follow-up are needed to test low-level laser therapy efficacy.

Acknowledgments The authors would like to thank all the volunteers and the financial support of the Sao Paulo Research Foundation (FAPESP grant nos. 2009/11284-4 to TMO). The authors would like to thank Daniele Santi Ceolin and Patrícia De Sá Mortágua Gemino for their excellent laboratorial assistance.

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

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