DOI: 10.1111/php.12155

Photochemistry and Photobiology, 2014, 90: 201–206

Effects of Laser Irradiation on Pulp Cells Exposed to Bleaching Agents

Adriano F. Lima[1,2], Fernanda G. Basso[3], Ana P. D. Ribeiro[4], Vanderlei S. Bagnato[5], Josimeri Hebling[6], Giselle M. Marchi[1] and Carlos A. de Souza Costa*[7]

[1]Department of Restorative Dentistry, Piracicaba Dental School, University of Campinas—UNICAMP, Piracicaba, Brazil
[2]Department of Restorative Dentistry, Nove de Julho University, Sao Paulo, Brazil
[3]Department of Pathology, Piracicaba Dental School, University of Campinas—UNICAMP, Piracicaba, Brazil
[4]Department of Operative Dentistry, University of Brasília-UnB, Brasilia, Federal District, Brazil
[5]Physics Institute of São Carlos, USP—University of São Paulo, São Carlos, Brazil
[6]Department of Orthodontics and Pediatric Dentistry, Araraquara School of Dentistry, Univ. Estadual Paulista (UNESP), Araraquara, Brazil
[7]Department of Physiology and Pathology, Araraquara School of Dentistry, Univ. Estadual Paulista (UNESP), Araraquara, Brazil

Received 29 April 2013, accepted 25 July 2013, DOI: 10.1111/php.12155

ABSTRACT

The aim of this study was to evaluate the effect of low-level laser therapy (LLLT) on odontoblast-like cells exposed to a bleaching agent. Mouse dental papilla cell-23 cells were seeded in wells of 24-well plates. Eight groups were established according to the exposure to the bleaching agent and LLLT (0, 4, 10 and 15 J cm^{-2}). Enamel–dentin disks were adapted to artificial pulp chambers, which were individually placed in wells containing Dulbecco's modified Eagle's medium (DMEM). A bleaching agent (35% hydrogen peroxide [BA35%HP]) was applied on enamel (15 min) to obtain the extracts (DMEM + BA35%HP components diffused through enamel/dentin disks). The extracts were applied (1 h) to the cells, and then subjected to LLLT. Cell viability (Methyl tetrazolium assay), alkaline phosphatase (ALP) activity, as well as gene expression of ALP, fibronectin (FN) and type I collagen, were evaluated. The bleaching procedures reduced the cell viability, ALP activity and gene expression of dentin proteins. Laser irradiation did not modulate the cell response; except for FN, as LLLT decreased the gene expression of this protein by the cells exposed to the BA35%HP. It can be concluded that BA35%HP decreased the activities of odontoblasts that were not recovered by the irradiation of the damaged cells with low-level laser parameters tested.

INTRODUCTION

Tooth bleaching as an esthetic treatment has been widely studied in recent decades, and particular attention has been paid to its toxicity to pulp cells (1–7). Reactive oxygen species (ROS) released from bleaching agents are capable of diffusing through enamel to interact with dark molecules present in dentin substrate, promoting tooth bleaching (8). Concomitantly, these toxic products can reach the pulp chamber (9) to cause cell damage (2,3).

The toxic effects promoted by tooth-bleaching treatments have been demonstrated in both *in vitro* (2,3,10) and *in vivo* studies (1,6) in which the authors showed a range of reactions, from mild pulp inflammation on bleached premolars (6) to partial necrosis of coronal pulp tissue in mandibular human incisors (1). According to previous *in vitro* studies, low concentrations of bleaching agents cause severe damage to cultured pulp cells, such as macrophages (11), fibroblasts (5,10) and odontoblasts (2–4). The rationale for the use of odontoblast-like cell lineages for evaluation of the *in vitro* cytotoxicity of dental materials is based on the fact that odontoblasts are the first pulp cells to be reached by subproducts released from dental products capable of diffusing through enamel and dentin (2,4) to cause cell damage.

In the last few years, low-level laser therapy (LLLT) have shown promising results related to the modulation of cell responses, such as increased ATP synthesis (12), osteoblast proliferation (13) and alkaline phosphatase (ALP) activity in odontoblast-like cells (14), as well as cell metabolism in fibroblasts exposed to bleaching agents (5). The objective of LLLT applied after tooth-bleaching procedures is to influence, in a positive way, the pulp cell responses, improving the healing of this specific connective tissue damaged by toxic components released from bleaching agents. The LLLT has been indicated to treat dental hypersensitivity, which is the side-effect most frequently observed in patients during and even after tooth-bleaching therapies (15,16). However, only scarce data concerning the efficacy of LLLT on biomodulation of pulp cells previously exposed to bleaching agents are available (5,17). Therefore, the aim of this study was to evaluate the effect of LLLT on odontoblast-like mouse dental papilla cell (MDPC)-23 cells immediately after exposure to components of a bleaching agent capable of diffusing across enamel and dentin. Cell metabolism, ALP activity and gene expression of dentin proteins (type I collagen [COL-I], fibronectin [FN], and ALP) were assessed.

MATERIALS AND METHODS

Cell culture. Immortalized cells of the MDPC-23 cell line (18,19), kindly provided by Dr. Carl T. Hanks and Dr. Jacques E. Nör, from the University of Michigan, Ann Arbor, USA, were cultured in Dulbecco's modified Eagle's medium (Sigma Chemical Co., St. Louis, MO). The cells were subcultured every 3 days to obtain adequate numbers, and then

*Corresponding author email: casouzac@foar.unesp.br (Carlos A. de Souza Costa)

were seeded (12 500 cells cm^{-2}) in 24-well dishes (Costar Corp., Cambridge, MA). The cells were maintained for 5 days in a humidified incubator (Isotemp, Fisher Scientific, Pittsburgh, PA) with 5% CO_2 and 95% air at 37°C.

Preparation of the artificial pulp chamber (APC). In this study, we used 208 enamel/dentin disks (dentin thickness = 1.6 mm; enamel thickness = 1.5 mm; diameter = 5 mm), obtained from bovine incisors. To obtain the disks, we cleaned the incisors, stored them in a thymol solution (0.1%) and analyzed them by light microscopy (20×, Meiji 2000; Meiji Techno, Saitama, Japan). Teeth with hypoplasia, enamel cracks or other morphological alterations were excluded from the study.

The teeth were sectioned with the use of a metallographic precision saw (ISOMET 1000; Buehler Ltd., Lake Bluff, IL) to obtain a block containing both enamel and dentin (10 mm width × 10 mm length). The specimens were then rounded with a high-speed, water-cooled cylindrical diamond bur (1095; KG Sorensen, Barueri, Brazil) to obtain standardized disks (5.0 mm of diameter). The dentin surfaces of the disks were wet-ground by means of abrasive papers (SiC #600 and #1200—Arotec Ind. Com. Ltda., Cotia, Brazil) to obtain a standardized thickness of 1.6 mm. The dentin thickness used in this study was standardized as 1.6 mm, similar to the dentin thickness present in the human central incisors (1,20), main target of the bleaching procedures. The enamel was not grounded to maintain the normal structure characteristics of the enamel surface. All these procedures were performed aiming to mimic the clinical situation of tooth bleaching. The smear layer was removed with 0.5 N ethylenediaminetetraacetic acid (Mallinckrodt Baker Inc., Paris, KY) solution, pH 7.2, for 30 s (21), and the disks were then rinsed thoroughly with sterile deionized water for 60 s.

After these procedures, the disks were individually adapted to an APC (22), with their enamel sides facing upward to receive the bleaching treatments. The APCs were sterilized and randomly divided into eight experimental and control groups (Table 1).

Bleaching treatment and laser irradiation. For the bleaching treatment, the APCs were individually placed in 24-well plates containing 1 mL of culture medium without fetal bovine serum (FBS). Each enamel surface was washed with distilled water and dried with absorbent papers, before the bleaching protocol was undertaken. The bleaching gel with 35% hydrogen peroxide (HP—Whiteness HP; FGM, Joinville, Brazil) was used according to the manufacturer's instructions. The bleaching gel used is presented in two bottles, one containing HP and the other one a thickener agent. To perform the bleaching, the HP and the thickener were mixed (3:1) to obtain a homogeneous solution. Then, the bleaching agent was applied on the enamel surface for 15 min.

The agent was then aspirated, and the enamel surface was gently washed with distilled water. After 45 min, a 500-μL quantity of the obtained extract (subproducts diffused through the dental structure + culture medium) was applied to the odontoblast-like cells (1 h).

After this period, the extracts were removed, new culture medium supplemented with FBS was applied and laser irradiation was performed. The laser device used in this study was an indium gallium arsenide phosphide (InGaAsP) diode laser prototype (LASERTable; 780 ± 3 nm wavelength, 0.025 W maximum power output, area of irradiation was 2 cm^2). LLLT was delivered from the bottom of the culture plates, where cells were previously seeded and treated or not with HP (2,17,23). Each well was irradiated individually at different energy doses (4, 10, and 15 J cm^{-2}) according to the established groups. The energy doses were selected according to results from previous studies demonstrating that LLLT at selected parameters could improve cell metabolism (14). Irradiation times were established at 300, 800 and 1200 s, for 4, 10 and 15 J cm^{-2}, respectively (1).

Twenty-four hours after irradiation, the evaluations were performed. All experiments were carried out in triplicate.

Cell viability analysis (Methyl tetrazolium assay). After the bleaching treatment and laser irradiation (Table 1), 10 wells were used for the evaluation of cell viability (n = 10) by the cytochemical demonstration of succinic dehydrogenase activity, as described previously by Mosmann (24) and used in several current studies (2,3,25,26). The absorbance values of each group were transformed into percentages representing cell viability. The negative control (without any treatment) was defined as 100% cell viability.

ALP activity. Ten wells *per* group (n = 10) were used for the evaluation of ALP activity by means of a colorimetric endpoint assay (ALP Kit; Labtest Diagnóstico S.A., Lagoa Santa, Brazil). Thymolphthalein monophosphate is a phosphoric acid ester substrate that is hydrolyzed by ALP and releases thymolphthalein, giving a bluish color to the solution. The intensity of the resulting color is directly proportional to the enzymatic activity and was analyzed by spectrophotometry, as described and used in other studies (26,27).

Gene expression of dentin proteins by real-time polymerase chain reaction (qPCR). Six wells were used for the evaluation of the gene expression of dentin proteins after the different treatments (n = 6). Protocols of RNA extraction and cDNA synthesis were performed as described by Basso *et al.* (23) and are briefly described here.

RNA extraction and cDNA synthesis: Twenty-four hours after the final irradiation, the expression of dentin proteins was evaluated by a two-step qPCR. RNA isolation was performed by the Trizol method (Invitrogen, Carlsbad, CA), and cDNA was synthesized from each RNA sample by the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) as previously described (22).

qPCR: After cDNA synthesis, the gene expression of ALP, COL-I and FN was evaluated by qPCR. For each gene, specific primers were synthesized from the mRNA sequence (Table 2). The reactions were prepared with standard reagents for real-time PCR (Syber Green Master Mix [Applied Biosystems]). qPCR reactions were performed with the Step One Plus System (Applied Biosystems) at each amplification cycle, and analyzed with Step One Software 2.1 (Applied Biosystems). Results were expressed in cycle threshold (CT) values, which represent the number of PCR cycles necessary for the fluorescent signal to reach the detection threshold. The individual results expressed in CT values were normalized according to the expression of the selected endogenous reference gene, beta-actin. The mRNA concentrations of each target gene were then analyzed statistically.

Statistical analysis. The normality of the results (Shapiro–Wilk test) and homogeneity of variances (Levene's test) of the data obtained were evaluated for all analysis. As the results of cell metabolism and ALP activity did not present a normal distribution, the data were statistically analyzed by a nonparametric test (Kruskal–Wallis and Dunn's tests; α = 0.05). The results of dentin protein gene expression (real-time PCR) were analyzed by two-way ANOVA and Tukey's test (α = 0.05).

Table 1. Experimental and control groups according to laser irradiation and bleaching treatment.

Groups	Bleaching treatment (HP 35%)	Dose (J cm^{-2})	Laser	Number of applications
G1 control	Absent	0	Absent	0
G2 control	Present	0	Absent	0
G3	Absent	4	Present	1
G4	Absent	10	Present	1
G5	Absent	15	Present	1
G6	Present	4	Present	1
G7	Present	10	Present	1
G8	Present	15	Present	1

RESULTS

Cell viability (Methyl tetrazolium)

The bleaching treatment used in this study caused a significant reduction in MDPC-23 viability (40–60%) (Kruskal–Wallis and

Table 2. Primer sequences in gene expression analysis with real-time PCR.

Genes	Primer sequences
Col-I	Forward—5′ ACG TCC TGG TGA AGT TGG TC 3′
	Reverse—5′ CAG GGA AGC CTC TTT CTC CT 3′
ALP	Forward—5′ GCT GAT CAT TCC CAC GTT TT 3′
	Reverse—5′ CTG GGC CTG GTA GTT GTT GT 3′
FN	Forward—5′ CTG GGG TCT ACT CCA CCG AA 3′
	Reverse—5′ GCC AAG ATT CAG AGA CCC GG 3′
β-Actin	Forward—5′ AGC CAT GTA CGT AGC CAT CC 3′
	Reverse—5′ CT CTC AGC TGT GGT GGT GAA 3′

Dunn's tests, $P < 0.05$) (Fig. 1). Laser irradiation at all tested parameters was not capable of reversing the cytotoxic effects caused by the bleaching treatment. Nevertheless, a higher reduction in cell viability occurred in those cells exposed to the bleaching agent and irradiated at 15 J cm^{-2} (G8) compared with that in the other groups exposed to bleaching ($P < 0.05$). The groups without exposure to the bleaching agent presented similar levels of cell metabolism, regardless of laser irradiation.

ALP activity

Laser irradiation was not capable of promoting any alteration in ALP activity in the groups with or without exposure to the bleaching agents (Fig. 2). All groups presented statistically similar results compared with the control groups (without irradiation). However, the bleaching treatment caused ALP activity reduction, regardless of laser application ($P < 0.05$).

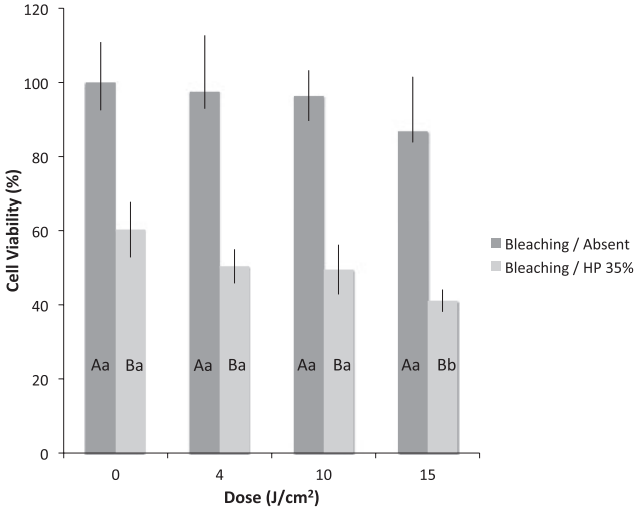

Figure 1. Cell viability of the odontoblasts according to the experimental and control groups. Different letters indicate statistically significant differences. Capital letters denote the bleaching treatment, and lower-case letters denote the energy doses (Kruskal–Wallis and Dunnet tests, $\alpha = 0.05$).

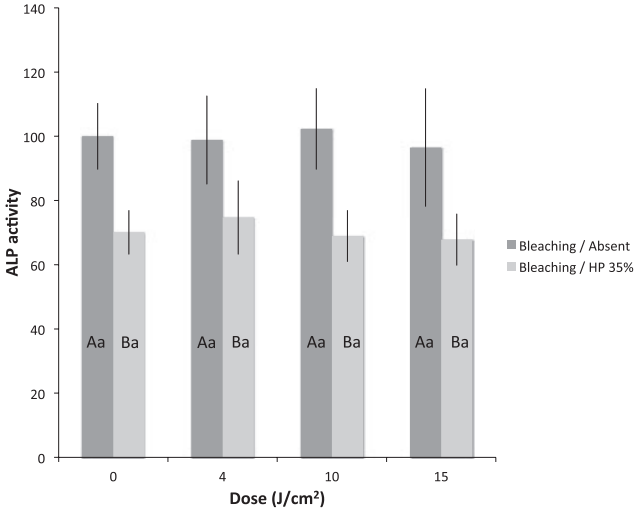

Figure 2. Graphic presentation of the alkaline phosphatase activity as a function of the different treatments. Different letters indicate statistically significant differences. Capital letters denote the bleaching treatment, and lower-case letters denote the energy doses (Kruskal–Wallis and Dunnet tests, $\alpha = 0.05$).

Gene expression of dentin proteins (real-time PCR)

In evaluations of the gene expression of ALP and COL-I, only the variable "bleaching treatment" presented a statistically significant difference (two-way ANOVA, $P < 0.001$). The expression of both genes was reduced after cells were exposed to the bleaching agents (Figs. 3 and 4). Moreover, the laser did not modulate the expression of both genes evaluated.

The interaction between the main variables ("bleaching treatment" and "laser irradiation") was significant for FN expression (two-way ANOVA, $P = 0.0034$). The negative influence of the bleaching treatment was observed for this gene expression, as

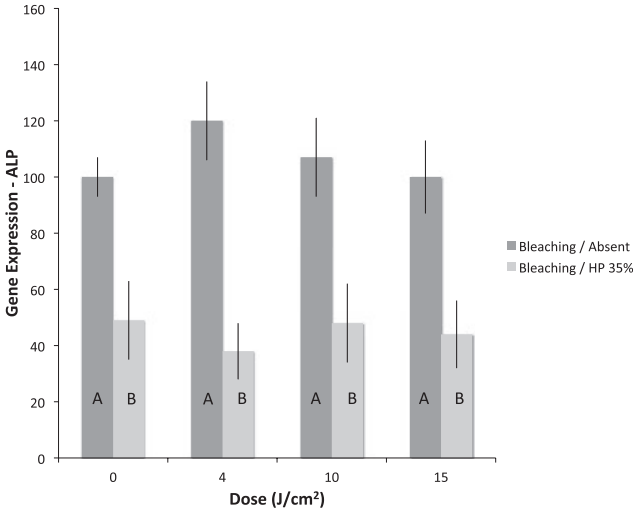

Figure 3. Gene expression of alkaline phosphatase after the treatments respective to each group. Different letters indicate statistically significant differences (Tukey's test, $\alpha = 0.05$).

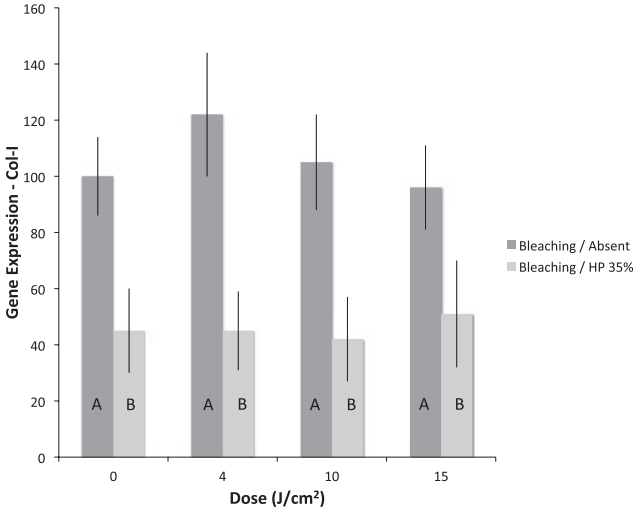

Figure 4. Gene expression of Col-I after the different protocols tested. Different letters indicate statistically significant differences (Tukey's test, $\alpha = 0.05$).

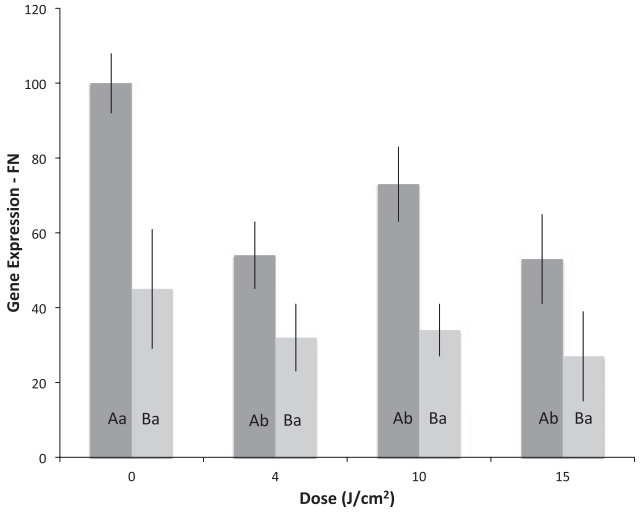

Figure 5. Gene expression of fibronectin according to the experimental and control groups. Different letters indicate statistically significant differences. Capital letters denote the bleaching treatment, and lower-case letters denote the energy doses (Tukey's test, $\alpha = 0.05$).

was previously observed for other genes (Fig. 5). Regarding the effect of laser irradiation, this therapy was not capable of reversing the negative effects observed for the cells exposed to bleaching subproducts. Indeed, laser irradiation decreased FN expression in those groups in which the cells were not exposed to bleaching agents.

DISCUSSION

Although bleaching therapy is an esthetic treatment widely performed in clinical practice, several negative side effects have been demonstrated (1–3,22,28). The affirmation "safe and noninvasive treatment" commonly used to describe this esthetic procedure has lately been called into question. As previously demonstrated, even at low concentrations, bleaching agents can cause damage to dental pulp cells (3,22), and previous results corroborate those of this study, in which we demonstrated the deleterious effects of tooth-bleaching agents on the viability of odontoblast-like cells.

The cell damage promoted by bleaching agents occurs due to the ROS released from the bleaching gel and diffused through the hard dental structures. These toxic oxygen species react with the unsaturated fatty acids of the plasmatic membrane (29), increasing its permeability as well as causing activation of endonucleases and proteases, and even apoptosis (cell death) (30). The ROS diffused through enamel and dentin reduces not only cell viability but also ALP activity. These highly reactive free radicals interact with the protein structure of ALP, breaking down the amino acid sequence and causing protein fragmentation (31). With the significant reduction in cell viability, the toxicity promoted by the bleaching agent decreased the cell activity of, and negatively influenced the gene expression by, cultured odontoblast-like cells, as observed in the real-time PCR results obtained. Analysis of these data indicates that several cell functions can be jeopardized by the exposure of pulp cells to the subproducts released from bleaching agents.

Thus, alternative therapies should be evaluated to reduce the damage caused by bleaching agents to pulp cells, and low-level laser therapy seems to be a promising option. According to previous studies, interesting results have been obtained regarding the influence of laser irradiation on cell differentiation, reduction in inflammatory reactions and tissue repair (32–36). Therefore, LLLT irradiation may be considered as a potential adjuvant therapy for the modulation of dental pulp cell responses to injury caused by bleaching treatment. Laser irradiation can modulate cell responses by acting in the mitochondria, increasing the levels of ATP and intracellular calcium (37). This kind of therapy may cause the dissociation of nitric oxide from the catalytic center of the cytochrome C oxidase (38). In addition, it has been demonstrated that laser irradiation is capable of increasing the viability of pulp fibroblasts previously exposed to bleaching agents (5). With regard to the odontoblast-like cells exposed to bleaching agents, it was demonstrated that the same LLL parameters used in this study increased ALP activity in cells after being irradiated three times (17). However, as shown in a previous study carried by Lima *et al.* (17), no modulation of cell viability was observed. In addition, the authors demonstrated that the cell metabolism of cultured odontoblasts exposed to bleaching agents was not affected by laser irradiation, except at the energy dose of 15 J cm^{-2}, at which cell metabolism was decreased. This reduced cell metabolism can be explained by the phenomenon known as "biphasic dose response" or "hormesis," according to which low light doses can cause positive effects on cell metabolism, and high energy doses may inhibit some cell functions, which can be in some cases, a negative result (39). The reduction in cell metabolism after laser irradiation with 15 J cm^{-2} was observed only for the group also exposed to the bleaching agents. This result suggests that the previous aggression caused by the bleaching agent increased the susceptibility of the cells to the negative effects on metabolism promoted by laser irradiation with 15 J cm^{-2}, causing a greater reduction in cell viability.

In addition to cell viability, the ALP activity and gene expression of proteins associated with the synthesis and mineralization of dentin, such as COL-I, ALP and FN, were also evaluated to verify whether laser therapy would be able to modulate cell responses to the subproducts released from the bleaching agents, with the possible formation of postirradiation dentin matrix. FN is a protein involved in the processes of adhesion, proliferation and cell differentiation (40). In addition, the presence of this protein is associated with tissue mineralization and dentin formation (41). The irradiation protocols tested were not capable of modulating the cell metabolism, as well as the ALP activity and gene expression, of dentin proteins, except for FN. For this last protein, laser irradiation promoted a reduction in gene expression, only for the cells not exposed to the bleaching agents. This data may be explained by the energy doses used in this study, which could be high enough to reduce the gene expression of FN by the cultured odontoblast-like cells, even when they were not exposed to the bleaching agent. However, these effects were observed only for the FN expression and cell viability.

Based on the results obtained, it may be hypothesized that a single laser irradiation, using the parameters tested, was not capable to promote cell effects immediately against the aggression caused by bleaching agents. Other parameters such as the wavelength used, potency of the laser irradiation and the doses tested can be responsible for the results obtained, as different cell responses can be obtained after changes in any parameters cited. (39,42) Nevertheless, due to the efficacy of this therapy in inflammation reduction and tissue repair (32,33,35,36), as well

as on the stimulation of fibroblast metabolism (5), and the increased ALP activity produced by odontoblasts exposed to dental materials such as bleaching agents (17), laser irradiation represents an interesting alternative therapy for the positive modulation of pulp tissue responses to aggressive stimuli. The irradiation parameters used in this study were based on a prior study, in which the authors demonstrated promising results using high laser energy doses on the same cell line used in this investigation (36). On the other hand, different from Oliveira *et al.* (36), in the present laboratorial study the authors induced a specific cellular stress using toxic components released from bleaching agents. Nevertheless, several studies have obtained positive results applying low energy densities to different cell lines (23,43–45). However, the definition of biostimulating parameters represents a challenge, and, therefore, other parameters should be evaluated to establish the optimum power and energy doses to be used in such situations.

CONCLUSIONS

Based on the results obtained, the following can be concluded:

1 The bleaching treatment caused a reduction in odontoblast metabolism, as well as in the activity of ALP and the gene expression of COL-I, ALP and FN.
2 Laser therapy, according to the evaluated parameters and a single irradiation, was not capable of influencing the cell metabolism, ALP activity and gene expression of ALP and COL-I after 24 h of the irradiation.
3 A single laser irradiation promotes an inhibitory effect on FN gene expression on odontoblasts without exposure to bleaching agents, in all doses evaluated after 24 h of the irradiation.

Acknowledgements—This work is part of a thesis submitted to Piracicaba Dental School in partial fulfillment of the requirements for the PhD degree to Adriano Fonseca Lima. This study was supported by the Fundacão de Amparo a Pesquisa do Estado de São Paulo—FAPESP (Grants: 2009/08992-7-8 and 2010/00645-3), and Conselho Nacional de Desenvolvimento Cientőfico e Tecnológico—CNPq (Grants: 301291/2010-1).

REFERENCES

1. Costa, C. A., H. Riehl, J. F. Kina, N. T. Sacono and J. Hebling (2010) Human pulp responses to in-office tooth bleaching. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **109**, e59–e64.
2. Dias Ribeiro, A. P., N. T. Sacono, F. C. Lessa, I. Nogueira, C. R. Coldebella, J. Hebling and C. A. de Souza Costa (2009) Cytotoxic effect of a 35% hydrogen peroxide bleaching gel on odontoblast-like MDPC-23 cells. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **108**, 458–464.
3. de Lima, A. F., F. C. Lessa, M. N. Gasparoto Mancini, J. Hebling, C. A. de Souza Costa and G. M. Marchi (2009) Cytotoxic effects of different concentrations of a carbamide peroxide bleaching gel on odontoblast-like cells MDPC-23. *J. Biomed. Mater. Res. B Appl. Biomater.* **90**, 907–912.
4. Lima, A. F., F. C. Lessa, J. Hebling, C. A. de Souza Costa and G. M. Marchi (2010) Protective effect of sodium ascorbate on MDPC-23 odontoblast-like cells exposed to a bleaching agent. *Eur. J. Dent.* **4**, 238–244.
5. Dantas, C. M., C. L. Vivan, L. S. Ferreira, P. M. Freitas and M. M. Marques (2010) In vitro effect of low intensity laser on the cytotoxicity produced by substances released by bleaching gel. *Braz. Oral Res.* **24**, 460–466.
6. Robertson, W. D. and R. C. Melfi (1980) Pulpal response to vital bleaching procedures. *J. Endod.* **6**, 645–649.
7. Kina, J. F., C. Huck, H. Riehl, T. C. Martinez, N. T. Sacono, A. P. Ribeiro and C. A. Costa (2010) Response of human pulps after professionally applied vital tooth bleaching. *Int. Endod. J.* **43**, 572–580.
8. Kawamoto, K. and Y. Tsujimoto (2004) Effects of the hydroxyl radical and hydrogen peroxide on tooth bleaching. *J. Endod.* **30**, 45–50.
9. Gokay, O., A. Mujdeci and E. Algin (2005) In vitro peroxide penetration into the pulp chamber from newer bleaching products. *Int. Endod. J.* **38**, 516–520.
10. Hanks, C. T., J. C. Fat, J. C. Wataha and J. F. Corcoran (1993) Cytotoxicity and dentin permeability of carbamide peroxide and hydrogen peroxide vital bleaching materials, in vitro. *J. Dent. Res.* **72**, 931–938.
11. Asfora, K. K., C. Santos Mdo, M. A. Montes and C. M. de Castro (2005) Evaluation of biocompatibility of sodium perborate and 30% hydrogen peroxide using the analysis of the adherence capacity and morphology of macrophages. *J. Dent.* **33**, 155–162.
12. Karu, T., L. Pyatibrat and G. Kalendo (1995) Irradiation with He-Ne laser increases ATP level in cells cultivated in vitro. *J. Photochem. Photobiol. B* **27**, 219–223.
13. Khadra, M., S. P. Lyngstadaas, H. R. Haanaes and K. Mustafa (2005) Effect of laser therapy on attachment, proliferation and differentiation of human osteoblast-like cells cultured on titanium implant material. *Biomaterials* **26**, 3503–3509.
14. Oliveira, C. F., F. G. Basso, E. C. Lins, C. Kurachi, J. Hebling, V. S. Bagnato and C. A. D. Costa (2010) Increased viability of odontoblast-like cells subjected to low-level laser irradiation. *Laser Phys.* **20**, 1659–1666.
15. Kossatz, S., A. P. Dalanhol, T. Cunha, A. Loguercio and A. Reis (2011) Effect of light activation on tooth sensitivity after in-office bleaching. *Oper. Dent.* **36**, 251–257.
16. Reis, A., A. P. Dalanhol, T. S. Cunha, S. Kossatz and A. D. Loguercio (2011) Assessment of tooth sensitivity using a desensitizer before light-activated bleaching. *Oper. Dent.* **36**, 12–17.
17. Lima, A. F., A. P. Ribeiro, F. G. Basso, V. S. Bagnato, J. Hebling, G. M. Marchi and C. A. de Souza Costa (2013) Effect of low-level laser therapy on odontoblast-like cells exposed to bleaching agent. *Lasers Med. Sci.* (In press, DOI: 10.1007/s10103-013-1309-2)
18. Hanks, C. T., Z. L. Sun, D. N. Fang, C. A. Edwards, J. C. Wataha, H. H. Ritchie and W. T. Butler (1998) Cloned 3T6 cell line from CD-1 mouse fetal molar dental papillae. *Connect. Tissue Res.* **37**, 233–249.
19. Sun, Z. L., D. N. Fang, X. Y. Wu, H. H. Ritchie, C. Begue-Kirn, J. C. Wataha, C. T. Hanks and W. T. Butler (1998) Expression of dentin sialoprotein (DSP) and other molecular determinants by a new cell line from dental papillae, MDPC-23. *Connect. Tissue Res.* **37**, 251–261.
20. Chandler, N. P. (1989) The radiographic assessment of pulp size: Validity and clinical implications. *N. Z. Dent. J.* **85**, 23–26.
21. Jacques, P. and J. Hebling (2005) Effect of dentin conditioners on the microtensile bond strength of a conventional and a self-etching primer adhesive system. *Dent. Mater.* **21**, 103–109.
22. Lima, A. F., F. C. Lessa, M. N. Mancini, J. Hebling, C. A. Costa and G. M. Marchi (2010) Transdentinal protective role of sodium ascorbate against the cytopathic effects of H_2O_2 released from bleaching agents. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **109**, e70–e76.
23. Basso, F. G., C. F. Oliveira, C. Kurachi, J. Hebling and C. A. Costa (2013) Biostimulatory effect of low-level laser therapy on keratinocytes in vitro. *Lasers Med. Sci.* **28**(2), 367–374.
24. Mosmann, T. (1983) Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **65**, 55–63.
25. de Mendonca, A. A., P. P. Souza, J. Hebling and C. A. Costa (2007) Cytotoxic effects of hard-setting cements applied on the odontoblast cell line MDPC-23. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **104**, e102–e108.
26. de Mendonca, A. M., C. F. de Oliveira, J. Hebling and C. A. Costa (2011) Influence of the activation mode of a self-etch resin-based luting cement upon the metabolism of odontoblast-like cells. *Am. J. Dent.* **24**, 233–238.
27. Oliveira, C. F., J. Hebling, P. P. C. Souza, N. T. Sacono, F. R. Lessa, R. F. Z. Lizarelli and C. A. S. Costa (2008) Effect of low-level laser irradiation on odontoblast-like cells. *Laser Phys. Lett.* **5**, 680–685.

28. Fugaro, O. J., J. O. Fugaro, B. Matis, R. L. Gregory, M. A. Cochran and I. Mjor (2005) The dental pulp: Inflammatory markers and vital bleaching. *Am. J. Dent.* **18**, 229–232.
29. Halliwell, B. (2007) Biochemistry of oxidative stress. *Biochem. Soc. Trans.* **35**, 1147–1150.
30. Bellomo, G. and F. Mirabelli (1987) Oxidative stress injury studied in isolated intact cells. *Mol. Toxicol.* **1**, 281–293.
31. Koyama, I., M. Yakushijin, M. Goseki, T. Iimura, T. Sato, M. Sonoda, S. Hokari and T. Komoda (1998) Partial breakdown of glycated alkaline phosphatases mediated by reactive oxygen species. *Clin. Chim. Acta* **275**, 27–41.
32. Freire, M. R. S., D. Almeida, J. N. Santos and V. A. Sarmento (2011) Evaluation of bone repair after radiotherapy by photobiomodulation-an animal experimental study. *Laser Phys.* **21**, 958–964.
33. Queiroga, A. S., F. B. Sousa, J. M. S. Araujo, S. D. Santos, C. D. S. Sousa, T. C. Quintans, T. P. Almeida, C. F. W. Nonaka, L. V. Batista and F. A. Limeira (2008) Evaluation of bone repair in the femur of rats submitted to laser therapy in different wavelengths: An image segmentation method of analysis. *Laser Phys.* **18**, 1087–1091.
34. Zungu, I. L., D. Hawkins Evans and H. Abrahamse (2009) Mitochondrial responses of normal and injured human skin fibroblasts following low level laser irradiation–an in vitro study. *Photochem. Photobiol.* **85**, 987–996.
35. Laraia, E. M., I. S. Silva, D. M. Pereira, F. A. Dos Reis, R. Albertini, P. de Almeida, E. C. Leal Junior and P. de Tarso Camillo de Carvalho (2012) Effect of low-level laser therapy (660 nm) on acute inflammation induced by tenotomy of achilles tendon in rats. *Photochem. Photobiol.* **88**, 1546–50.
36. de Almeida, P., R. A. Lopes-Martins, S. S. Tomazoni, G. M. Albuquerque-Pontes, L. A. Santos, A. A. Vanin, L. Frigo, R. P. Vieira, R. Albertini, T. de Carvalho Pde and E. C. Leal-Junior (2013) Low-level laser therapy and sodium diclofenac in acute inflammatory response induced by skeletal muscle trauma: Effects in muscle morphology and mRNA gene expression of inflammatory markers. *Photochem. Photobiol.* **89**(2), 501–507.
37. Karu, T. I., L. V. Pyatibrat and N. I. Afanasyeva (2004) A novel mitochondrial signaling pathway activated by visible-to-near infrared radiation. *Photochem. Photobiol.* **80**, 366–372.
38. Sarti, P., M. Arese, A. Bacchi, M. C. Barone, E. Forte, D. Mastronicola, M. Brunori and A. Giuffre (2003) Nitric oxide and mitochondrial complex IV. *IUBMB Life* **55**, 605–611.
39. AlGhamdi, K. M., A. Kumar and N. A. Moussa (2012) Low-level laser therapy: A useful technique for enhancing the proliferation of various cultured cells. *Lasers Med. Sci.* **27**, 237–249.
40. Nakashima, M. (2005) Bone morphogenetic proteins in dentin regeneration for potential use in endodontic therapy. *Cytokine Growth Factor Rev.* **16**, 369–376.
41. Tziafas, D., N. Panagiotakopoulos and A. Komnenou (1995) Immunolocalization of fibronectin during the early response of dog dental pulp to demineralized dentine or calcium hydroxide-containing cement. *Arch. Oral Biol.* **40**, 23–31.
42. Moore, P., T. D. Ridgway, R. G. Higbee, E. W. Howard and M. D. Lucroy (2005) Effect of wavelength on low-intensity laser irradiation-stimulated cell proliferation in vitro. *Lasers Surg. Med.* **36**, 8–12.
43. Huang, Y. Y., K. Nagata, C. E. Tedford, T. McCarthy and M. R. Hamblin (2012) Low-level laser therapy (LLLT) reduces oxidative stress in primary cortical neurons in vitro. *J. Biophotonics* (In press, DOI: 10.1002/jbio.201200157)
44. Basso, F. G., T. N. Pansani, A. P. Turrioni, V. S. Bagnato, J. Hebling and C. A. de Souza Costa (2012) In vitro wound healing improvement by low-level laser therapy application in cultured gingival fibroblasts. *Int. J. Dent.* **2012**, 719452.
45. Pereira, L. B., D. T. Chimello, M. R. Ferreira, L. Bachmann, A. L. Rosa and K. F. Bombonato-Prado (2012) Low-level laser therapy influences mouse odontoblast-like cell response in vitro. *Photomed Laser Surg.* **30**, 206–213.