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# Laser irradiation did not increase the proliferation or the differentiation of stem cells from normal and inflamed dental pulp

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ABSTRACT

*Objective:* Low-level laser therapy (LLLT) has been reported to be responsible for promoting photostimulatory and photobiomodulatory effects *in vivo* and *in vitro*, stimulating cell growth, increasing cell metabolism, improving cell regeneration and invoking an anti-inflammatory response. This study was performed in order to investigate whether low-level laser therapy could increase the proliferation and differentiation potentials of hDPSC isolated from healthy dental pulps and from inflamed pulps.

*Design:* Human dental pulp stem cells (hDPSC) were isolated from normal and inflamed dental pulps from different patients. STRO-1-positive cells were isolated and irradiated with a red low-level laser (660 nm) in four different energy fluences (0.05, 0.30, 7 and 42 J/cm$^2$); the authors hypothesized that the first three fluences would promote biostimulatory effects, whereas the highest dose would induce antiproliferative effects. The two lower fluences were produced by irradiating the two higher fluences through a dentine disc, which was used to simulate a clinical condition. The proliferation and the cell odonto-osteogenic differentiation competence were compared.

*Results:* No statistically significant differences were observed between the proliferation rates and the relative productions of mineralized nodules compared to the respective controls, either for hDPSC from normal or inflamed dental pulps.

*Conclusions:* The irradiation with low-level InGaAlP red low-level laser (660 nm) in four different energy fluences (0.05, 0.30, 7 and 42 J/cm$^2$) potentiated neither proliferation nor odonto-osteogenic differentiation of hDPSC isolated from patients with normal and inflamed pulps.



## 1. Introduction

Human dental pulp stem cells (hDPSC), isolated from healthy teeth and expanded in culture, have shown multipotency. They have been studied for diverse tissue-regenerative purposes, not only in dentistry but also in various medical applications.[1–6]

When a dental pulp is diagnosed with irreversible pulpitis, the therapeutic indication is traditional endodontic treatment, which consists of removing the pulp tissue, chemomechanically preparing the pulp chamber and the root canals and

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filling the chamber and canals with inert and biocompatible materials. The inflamed vital tissue is, therefore, discarded.

Recently, stem cells were isolated from clinically compromised dental pulp with irreversible pulpitis. They have shown favourable proliferative potential and *ex vivo* odonto-osteogenic differentiation capacity and potent in tissue regeneration *in vivo*.[7,8]

Low-level laser therapy (LLLT) has been reported to be responsible for promoting photostimulatory and photobiomodulatory effects *in vivo* and *in vitro*, stimulating cell growth, increasing cell metabolism, improving cell regeneration and invoking an anti-inflammatory response.[9] A photobiomodulating effect was observed in teeth exposed to LLLT, which resulted in lower levels of pulpal pain compared to teeth exposed to the placebo treatment.[10] LLLT also accelerated dentine regeneration after dental-pulp exposure.[11,12] Additionally, it has been reported that LLLT may be used to increase the number of stem cells in culture, to induce differentiation into specific cell types or both.[13] It has been reported that hDPSC responds positively to laser phototherapy by improving the cell growth *in vitro*.[14]

Laser irradiation at low doses biomodulates cell activity by some postulated mechanisms. It is proposed that it excites molecules of the mitochondrial respiratory chain, intensifying the formation of a transmembrane electromechanical proton gradient in mitochondria. It enhances cell proliferation by increasing the release of calcium into the cytoplasm, which triggers mitosis; by causing a short-term rise in the intracellular pH, which triggers mitogenic signals in the cells and also by increasing ATP production.[15,16]

In a recent review of 32 studies on the subject, the following was concluded: (1) for biological effects, laser wavelengths may be red or near infrared (600–1200 nm); and (2) for biostimulatory effects, energy fluences between 0.05 and 10 J/cm$^2$ may induce cell proliferation, whereas energies greater than this value (>10 J/cm$^2$) may promote antiproliferative effects.[17]

Within this context, this study was performed in order to investigate whether low-level laser therapy could increase the proliferation and differentiation potentials of hDPSC isolated from healthy dental pulps and from inflamed pulps. The results of this study may be applicable to tissue engineering using hDPSC isolated from normal and inflamed dental pulp and to possible clinical LLLT in the modulation of pulpal responses under inflammatory conditions.

## 2. Materials and methods

### 2.1. Dental pulp tissues

Pulp tissues were obtained from the permanent teeth of patients (17–43 years of age) under approval of the Ethical Committee of Health Sciences Faculty of the University of Brasília (Brazil) (Project number 023/08) after informed consent. Normal pulp tissues were collected from 3 patients undergoing third molar extraction (N1–N3). These teeth were free of carious lesions. Inflamed dental pulps were obtained from 3 patients (I1–I3) with irreversible pulpitis that required treatment procedures to remove pulp tissue from the involved teeth. The diagnosis of irreversible pulpitis was determined by an endodontic specialist on the basis of clinical assessment, including history of spontaneous pain and intense, lingering pain to cold stimulus. The vitality of the pulp was confirmed on access. Teeth with completely necrotized pulp tissue were excluded.[7]

### 2.2. Cell culture

Dental pulp from normal and inflamed teeth was harvested as previously described.[7] To remove the dental pulp from extracted teeth, a longitudinal furrow was made using sterilized diamond discs without reaching the pulp tissue (KG Sorensen, ref: 7020). The teeth were fractured with a dental surgical elevator and pulp tissue was gently separated with a sterile dentinal excavator from the crown and root. Inflamed pulp tissues were collected from pulp chambers and root canals with a sterile endodontic file after complete exposure of pulp chamber. Normal and inflamed pulp tissues were transferred into sterile regular medium: α-minimum essential medium (α-MEM; Gibco, Gaithersburg, MD) supplemented with 10% foetal bovine serum (FBS; Gibco), 100 units/ml penicillin and 100 mg/ml streptomycin (Gibco). All pulp tissues were washed with α-MEM, digested with 3 mg/ml collagenase type I (Gibco) in supplemented medium for 60 min at 37 °C. After enzymatic digestion, cell suspension was washed three times by centrifugation (10 min at 750 × *g*) in culture medium and placed into 6-well plates. They were incubated at 37 °C in a 5% $CO_2$ and 80% humidity environment. Antibiotics (penicillin and streptomycin) were used in all washing, digesting buffer, and culture media to minimize bacterial contamination. Each sample of normal and inflamed pulp tissue was processed, cultured and evaluated separately in all experiments. The cells were maintained semiconfluently in order to prevent differentiation.

### 2.3. STRO-1-positive cell isolation

STRO-1 has been used as a stromal stem-cell surface marker.[18] To obtain enriched STRO-1-positive cells, the Dynabead (Invitrogen) positive isolation method was used according to the manufacturer's instructions, as previously described.[7]

### 2.4. Laser irradiation

Cell suspensions ($10^6$ cells/mL in 200 μL) in regular medium without phenol red (Gibco) were placed in individual sterile wells of 96-well, V-bottom plates (Greiner). Laser irradiation was conducted in the top of the well at a constant distance of 15 mm from the bottom of the plate, using an InGaAlP low-level red laser (660 nm, MMOptics, São Carlos, São Paulo, Brazil).

Experimental conditions used to determine the effect of laser irradiation on cell growth of both types of cells, from normal (N1–N3) and inflamed pulps (I1–I3) are summarized in Table 1. L10 and L60 correspond to cells that were irradiated with a constant power of 28 mW for 10 and 60 s, achieving two laser energy fluences of 7 and 42 J/cm$^2$, respectively. A laser