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Research Paper
Dental Implants

Low-level laser therapy improves peri-implant bone formation: resonance frequency, electron microscopy, and stereology findings in a rabbit model

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Abstract. Previous studies have reported positive effects of low-level laser therapy (LLLT) on bone healing. This study evaluated the effects of LLLT on peri-implant healing in vivo. Thirty-two rabbits had their mandibular left incisors removed, followed by immediate insertion of a dental implant into the fresh socket. Animals were assigned randomly to four groups: control (non-irradiated) or LLLT at three different doses per session: 5 J/cm^2, 10 J/cm^2, and 20 J/cm^2. A GaAlAs laser (830 nm, 50 mW) was applied every 48 h for 13 days, starting immediately after surgery. The implant stability quotient (ISQ) was measured using resonance frequency analysis upon implant insertion and immediately after death, 30 days after the last application. Tissues were prepared for scanning electron microscopy (SEM) and stereology. Variables measured were bone–implant contact (BIC) and bone neoformation within implant threads at three different sites. The results showed better ISQ for the 20 J/cm^2 group ($P = 0.003$). BIC values were significantly higher ($P < 0.05$) in the 20 J/cm^2 group, on both SEM and stereology. Bone area values were better in the 10 J/cm^2 ($P = 0.036$) and 20 J/cm^2 ($P = 0.016$) groups compared to the control group. Under these conditions, LLLT enhanced peri-implant bone repair, improving stability, BIC, and bone neoformation. The findings support and suggest parameters for the design of clinical trials using LLLT after implant placement.

Keywords: laser therapy; low-level; dental implants; osseointegration; scanning electron microscopy; histology.

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The rationale for the use of low-level laser therapy (LLLT) relies on its ability to exert, at the cellular level, biomodulatory effects on the molecular and biochemical processes that take place during intrinsic tissue repair.[1–10] Several in vivo and in vitro studies have suggested positive effects of LLLT on the tissue repair process, both in animal models and in culture media.[11–20] These therapeutic effects include the following: increased epithelial and fibroblast proliferation and enhanced collagen synthesis, thus speeding the process of repair; increased potential for bone remodelling and repair; restoration of nerve function after injury; normalization of hormonal function; immune regulation; reduced inflammation and oedema; modulation and relief of pain; and improved postoperative analgesia.[1–9] Even though dose is one of the most important parameters of laser therapy,[21] the data available are not sufficient to support the design of clinical studies.[11–20,22,23]

Preclinical studies have suggested that LLLT has beneficial effects on bone repair.[6,10,11] Regarding peri-implant bone healing after titanium implant placement,[12–15,24] previously published studies have shown more evident bone maturation[12,13,24] and increased bone–implant contact (BIC)[16] in LLLT-irradiated bone than in control groups. The main findings reported in the literature are summarized in Table 1.

The objective of this study was to assess the local effects of LLLT on the peri-implant healing process after implant placement in the rabbit mandible, immediately after mandibular incisor extraction, based on resonance frequency analysis (RFA), BIC, and bone neoformation area (BA) within implant threads, measured using scanning electron microscopy (SEM) and stereological analysis.

Materials and methods

Animals

The study sample comprised 32 male New Zealand rabbits (*Oryctolagus cuniculus*), weighing 3–4 kg and aged 3 months. The animals were allocated randomly to one of four different groups, with eight in each: three experimental groups treated with LLLT at different energy densities (5 J/cm^2, 10 J/cm^2, and 20 J/cm^2) and one non-irradiated control group. All animals received a solid diet and water ad libitum throughout the experiment and were housed under normal lighting, humidity, and temperature conditions in a climate-controlled environment. All animals underwent extraction of the mandibular left incisor followed by immediate placement of a dental titanium implant in the fresh socket.

Surgical protocol

Animals were anesthetized by intramuscular injection of ketamine hydrochloride (40 mg/kg) and xylazine hydrochloride (3 mg/kg). The area around the mandibular left incisor was prepared with 2% chlorhexidine digluconate and local infiltration of 0.5 ml lidocaine hydrochloride 2% with epinephrine 1:100,000. The mandibular left incisor was extracted with the aid of #5 paediatric extraction forceps. The fresh extraction socket was then drilled gradually, and a dental implant (3.25 mm diameter × 11.5 mm, NanoTite; BIOMET 3i, Florida, USA) placed in accordance with the manufacturer's instructions. Implant stability was measured using RFA, followed by placement of a cover screw. The socket was sutured with 4–0 nylon monofilament. While the animal was still under anaesthesia, the site of laser irradiation was shaved and the long axis of the implant marked on the skin with a surgical marker. At the end of the procedure, animals received analgesia and antimicrobial prophylaxis (Fig. 1). Perioperative procedures were performed by a veterinary physician. The authors performed the surgeries and LLLT procedures.

LLLT irradiation

Spot laser irradiation was performed using a gallium–aluminium–arsenide (GaAlAs) active medium infrared diode laser (wavelength 830 nm, power 50 mW), in continuous emission mode (Thera Lase; DMC Equipamentos, São Carlos, SP, Brazil), applied every 48 h over a 13-day intervention period for a total of seven applications. The first session was started immediately after surgery.

Energy density varied among the groups. The laser was applied holding the handpiece perpendicular to the basal bone of the mandible. Animals in the 5 J/cm^2 experimental group received two spot doses of 2.5 J/cm^2 per session, one point medial and one lateral to the long axis of the implant, as marked previously on the overlying skin, for a total dose of 5 J/cm^2 per session (index dose). Animals in the 10 J/cm^2 group received twice the index dose (5 J/cm^2 per point, for a total 10 J/cm^2 per session), and those in the 20 J/cm^2 group received four times the index dose (10 J/cm^2 per point, for a total 20 J/cm^2 per session).

Non-irradiated animals (control group) underwent sham irradiation, i.e., all the procedures performed in the experimental groups were also performed in the control group, but with the laser device unpowered (Table 2).

Death

On day 45 of the experiment (30 days after the last LLLT session), the animals were sedated (same protocol used for the surgical procedure) and killed with an overdose of 1% propofol (1 ml/kg) and 10%

Table 1. LLLT protocols described in previous studies evaluating peri-implant effects.

Author	Year	Type of light	Animal model	*n*	Wavelength (nm)	Power (mW)	Total dose (J/cm^2)	No. of sessions
Dörtbudak et al.[17]	2002	Red	Monkey	5	690	100	30	5
Pinheiro et al.[10]	2003	Infrared	Rabbit	14	830	10	602	7
Khadra et al.[24]	2004	Infrared	Rabbit	12	830	150	270	10
Lopes et al.[13]	2005	Infrared	Rabbit	14	830	10	602	7
Jakse et al.[12]	2007	Red	Rabbit	12	680	75	12	3
Kim et al.[22]	2007	Infrared	Mouse	20	830	96	40.32	7
Lopes et al.[14]	2007	Infrared	Rabbit	14	830	10	602	7
Pereira et al.[26]	2009	Infrared	Rabbit	12	780	70	367.5	7
Campanha et al.[11]	2010	Infrared	Rabbit	30	830	10	602	7
Maluf et al.[15]	2010	Infrared	Mouse	24	795	120	48	6

LLLT, low-level laser therapy.