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Infrared Laser Light Reduces Loading Time of Dental Implants: A Raman Spectroscopic Study

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ABSTRACT

Objective: **The aim of this study was to assess, through near-infrared Raman spectroscopy (NIRS), the incorporation of hydroxyapatite of calcium (CHA; ~960 cm^{-1})—on the healing bone around dental implants submitted or not to low-level laser therapy (LLLT) (λ830 nm).** ***Background Data:*** **The process of maturation of the bone is important for the success of dental implants, as it improves the fixation of the implant to the bone, allowing the wearing of a prosthesis. LLLT has been suggested as a mean of improving bone healing because of its biomodulatory capabilities.** ***Methods:*** **Fourteen rabbits received a titanium implant on the tibia; eight of them were irradiated with λ830-nm laser (seven sessions at 48-h intervals, 21.5 J/cm^2 per session, 10 mW, ϕ~0.0028 cm^2, 85 J/cm^2 treatment dose), and six acted as control. The animals were sacrificed at 15, 30, and 45 days after surgery. Specimens were routinely prepared for Raman spectroscopy. Twelve readings were taken on the bone around the implant.** ***Results:*** **The results showed significant differences in the concentration of CHA on irradiated and control specimens at both 30 and 45 days after surgery ($p < 0.001$).** ***Conclusion:*** **It is concluded that LLLT does improve bone healing, and this can be safely assessed by Raman spectroscopy.**

INTRODUCTION

In modern dentistry, implantology is accepted by patients as an effective prosthetic rehabilitation, as it restores the capacity of mastication, phonation, and esthetics.[1] It is known that success of dental implants depends on close contact between bone and implant.[2] During the healing process, immature bone is replaced by a mature type of bone, which incorporates inorganic components, with calcium hydroxyapatite (CHA[$Ca_{10}(PO_4)_6(OH)_2$]) being the most important one.[3] Mature bone is needed before the loading of the implants, as masticatory forces may displace loose ones. Usually, a waiting time of about 4–6 months is required for loading.[2] Different techniques such as grafts, osteoconductive biomaterials,[4] bone transplants,[5] and also the application of biostimulators such as ultrasound[6] have been tried in dentistry, with the ultimate aim of improving the quality of bone around dental implants.

Low-level laser therapy (LLLT) has been successfully used for improving bone healing in several conditions such as in alveolus of dental extraction,[7] in bone fractures,[8–10] during orthodontic treatments,[11] and in dental implant post-operations.[12] Previous reports on the use of LLLT before, during, and after the placement of dental implants[13,14] showed that irradiated subjects reported less pain and better tissue repair; these were attributed to both increased neo-vascularization and collagen deposition. In another study, better bone healing around dental implants was observed in animals irradiated with CW 40 mW λ830-nm diode laser, (ϕ~1 mm) with a total dose per session of 4.8 J/cm^2 at 45 and 60 days after the placement of the implant compared with the control group.[12]

Although microscopic examination and imaging are the most frequent methods for the assessment of bone healing around dental implants, these are unable to give information at the molecular level.[15] An alternative method that has been proposed as effective to assess tissues at the molecular level is near-infrared Raman spectroscopy (NIRS), which has been used on several non-invasive diagnostic applications of biological samples such as several types of cancers,[16] human coronary arteries,[17,18] blood analysis,[19] biocompatible implants, synthetic apatites, mineralization, teeth, and diseased bone,[15] in order to

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evaluate the microstructure of human cortical bone (osteon)[3] and to investigate the long-term effects on the surface microstructure of disks of CHA implanted inside articular capsules of mice.[20] In a recent study,[21] Raman spectroscopy was used to investigate the effects of LLLT (λ660 nm, 10 J/cm^2) on the healing of fractured bone of rats by monitoring the level of CHA.

In the present study, LLLT was used on the bone around dental implants inserted on the tibia of the rabbit; the level of incorporation of CHA was assessed by NIRS using specific Raman bands to characterize the presence of mineral on the bone, the phosphate symmetric stretch band (phosphate ν_1; ~960 cm^{-1}), which is a prominent marker.[22]

MATERIALS AND METHODS

Cylindrical titanium implants (ϕ2.6 mm; 6 mm long, Dent-Fix®, Cambuí-MG, Brazil) were inserted under general anesthesia (Acepromazine 1 mg/kg (Acepran® 0.2%, Univet S.A) and Butorfanol® 0.02 mL/kg (Lab. Strong Dodge Ltda); Zoletil® 50 mg, 15 mg/kg (Zolazepan and Thiletamine, Lab. Virbac S.A) in the right tibiae of 14 young male adult New Zealand rabbits (average weight 2 kg). A 4-cm-long incision was performed at the right tibia with a no. 15 scalpel blade. Skin and subcutaneous tissues were dissected down to the periosteum, which was gently sectioned, exposing the bone. Under refrigeration and using a low-speed drill (1200 rpm), a cavity was prepared at the tibia for the insertion of the implant. After the insertion of the implant, the periosteum was repositioned, and suturing was performed up to the skin (catgut® 3.0 and mononylon 3.0). All the animals received a single dose of Pentabiotico® (penicillin, streptomycin, 20,000 UI, Lab. Forte Dog de Ltda.) immediately after surgery. LLLT (λ830 nm, 10 mW, ϕ~0.0028 cm^2) was used on nine animals transcutaneously in four points around the implants at 48-h intervals (21.5 J/cm^2) during 15 days, resulting in an 85 J/cm^2 treatment dose; control subjects (n = 6) were submitted to a sham treatment following the same routine and acted as control. In order to standardize the location of irradiation, four tattoos were made on the skin using Nankin Ink around the borders of the implant immediately after surgery in order to allow irradiation to be carried out at the same point. The animals were sacrificed at 15, 30, and 45 days after the surgery with an overdose of general anesthetics. The specimens were removed and stored in liquid nitrogen for Raman spectroscopic analysis in order to minimize the growth of aerobic bacteria[23]; chemical fixation was not recommended due to fluorescence emissions from the fixative substances.[17] Prior to Raman study, the samples were longitudinally cut and warmed gradually to room temperature, and a few drops of saline were added to the surface during spectroscopic measurements. For Raman measurements, a Ti: sapphire laser (Spectra Physics, model 3900S) pumped by argon laser (Spectra Physics, model 2017S) provided near-infrared excitation. The Raman spectra of the bone healing around the implant were collected by a CCD detector (Princeton Instruments, model LN/CCD-1024-EHR1) cooled by liquid nitrogen (Fig. 1). The laser power used at the sample was 80 mW and spectral acquisition time 100 sec. Four points for measurement around the implants at the superior, medium, and inferior thirds

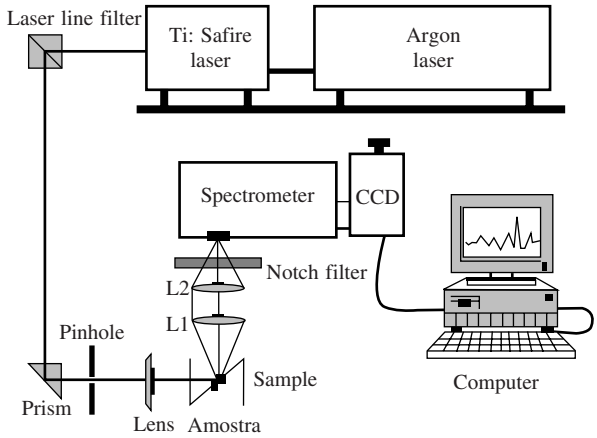

FIG. 1. Schematic diagram of the experimental setup for Raman spectroscopy measurements.

resulted in 12 readings of each implant and 168 total spectra (Fig. 2). The data were analyzed by MatLab® (The Mathworks, Inc.) software for calibration and background subtraction of the spectra. For calibration, the Raman spectrum of a solvent Indine with known peaks was used[23] due to its intense bands in the region of 700–1800 cm^{-1}. In order to remove the "fluorescence" from the original spectra, a fifth order polynomial fitting was performed. An average spectrum was generated for

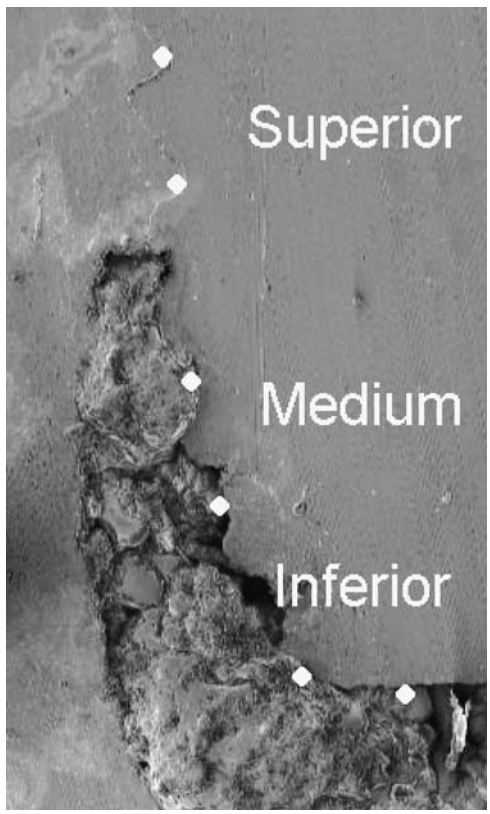

FIG. 2. Dental implant thirds (superior, medium, and inferior) by Raman analyzed in bone healing.

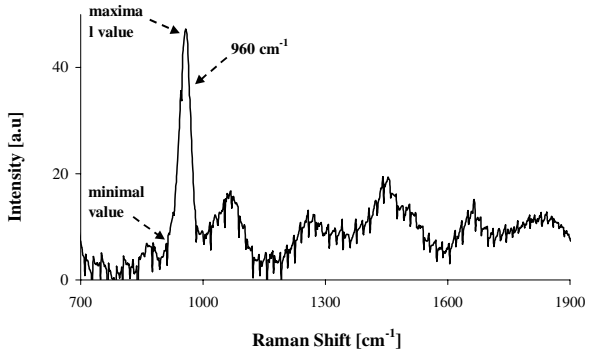

FIG. 3. Maximum and minimal intensities Raman values of the 960 cm^{-1}.

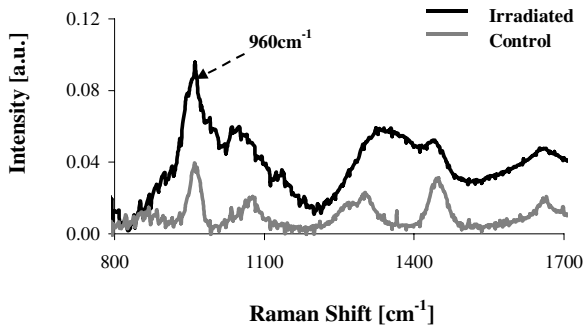

FIG. 5. Means of all Raman spectrum of phosphate ν_1 (960 cm^{-1}) at 30 days after surgery.

each time period (15, 30, and 45 days), and the mean value of the intensity of the peak (960 cm^{-1}) was determined by the difference between the maximum and minimal intensity measured (Fig. 3). This intensity is related to the concentration of CHA on the bone. Statistical analysis was performed using Instat® software. Kolmorogov and Smirnov test, *T* test, ANOVA, Turkey-Kramer test, or Mann-Whitney *U* test were used for the analysis.

RESULTS

The mean value of the 960 cm^{-1}(phosphate ν_1) peak of CHA of the healing bone of irradiated and control subjects at days 15, 30, and 45 can be seen in Figures 4–6. Figure 7 shows the deposition of CHA during the experimental period.

The results of the Raman spectroscopy carried out around the implant on control specimens at 15, 30, and 45 days were, respectively, 32 ± 3 SE, 46 ± 4 SE, and 43 ± 5 SE. On irradiated subjects, the results were as follows: 32 ± 2 SE, 70 ± 4 SE, and 73 ± 3 SE.

Statistical analysis of the results of the measurements of the concentrations of CHA at days 15, 30 and 45 showed that, at day 15, there was no significant difference between irradiated and control samples ($p > 0.05$). Up to the 45th day after surgery, the amount of CHA was significantly higher on irradiated subjects ($p < 0.001$, Fig. 7).

DISCUSSION

The successful use of Raman spectroscopy to assess the amount of CHA on bone has been previously reported under different conditions.[3,21,24,25] Bone healing of rabbits takes about 42 days, and in human, it takes from 4 to 6 months, when the bone becomes mature and resistant and capable of receiving loading without compromising the stability of the implant.[26] The results of the present study indicate early bone maturation on irradiated subjects due to increased deposition of CHA from day 30. Up to 15 days after surgery, there was no significant difference between irradiated and control subjects regarding the concentration of CHA. This may be a result of the fact that, during early stages of healing, the osteoblastic activity was chiefly proliferative and deposition started later, which resulted in the formation of immature bone, still poor in CHA.[26] From day 15, deposition of CHA was detectable in both groups, and from day 30, it was significantly higher in irradiated subjects. This represents the improved ability of more mature osteoblasts to secrete CHA in irradiated subjects, whereas in controls, cell proliferation was still occurring. Deposition of CHA represents bone maturation. The results of this study indicate that LLLT increased the concentration of CHA. Increased amount of CHA in bone is indicative of a more resistant bone.

The observed differences in the rate of deposition of CHA between irradiated and control subjects is probably due to the choice of a wavelength with higher penetration (λ830 nm) and

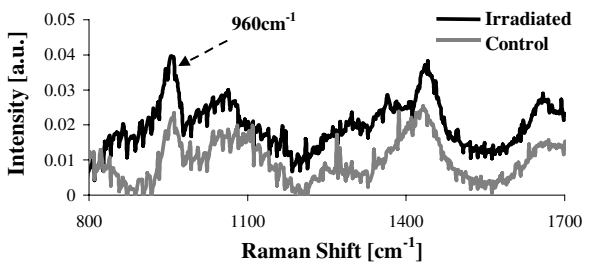

FIG. 4. Means of all Raman spectrum of phosphate ν_1 (960 cm^{-1}) at 15 days after surgery.

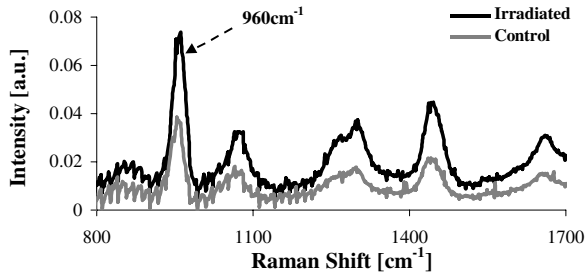

FIG. 6. Means of all Raman spectrum of phosphate ν_1 (960 cm^{-1}) at 45 days after surgery.

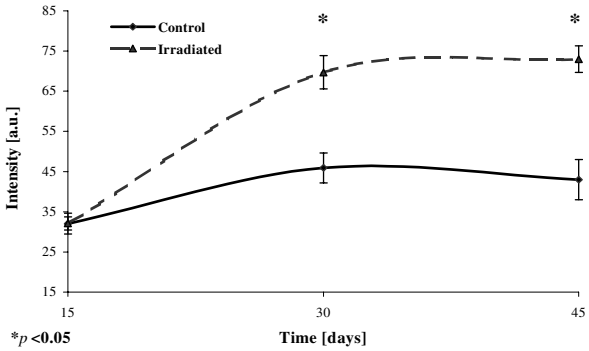

FIG. 7. Averages of the intensities of (phosphate $\nu_{1;}$ ~960 cm^{-1}) CHA in healing bone around the dental implants during the experimental time.

the ability to increase changes at cellular levels, such as improved ATP synthesis,[27] early osteoblastic differentiation,[12,28,29] and the release of growth factors.[30]

It is known that hydroxyapatite crystals are found on collagen fibers, within them, and in the matrix around. To initiate mineralization, high local concentrations of Ca^{2+} and PO^{43-} ions must be reached in order to induce their precipitation into amorphous calcium phosphate, leading to hydroxyapatite crystal formation. This is achieved by membrane-bound matrix vesicles, which originate by budding from the cytoplasmic process of the chondrocyte or the osteoblast, and it is deposited within the matrix during its formation. It is known that LLLT has the ability to stimulate cell proliferation, including of fibroblasts; this cell has the capacity to secrete collagen.

In the matrix, hydroxyapatite crystals have been observed, and they will grow in clusters, which later coalesce to completely calcify the matrix, filling the spaces between and within the collagen fibers. It is known that, during the many stages in bone healing, several cytokines and growth factors regulate matrix production. Various factors such as bone morphogenetic proteins, transforming growth factor–beta, and platelet-derived growth factor have been successfully used to augment healing in experimental models. Laser light also has positive effects on the release of several such mediators. Increased amounts of CHA may be positively correlated to bone mineral density, as higher intakes of calcium result in an increase in bone mineral density. However, is not known if higher amounts of CHA would interfere with attempts to strengthen bone, possibly by impairing magnesium absorption. This requires further clarification.

The reason why the effect of LLLT was not detectable until 30 days after surgery was probably due to the fact that, during early stages of bone healing, the cellular component is more prominent and more prone to be affected by LLLT. Later, bone matrix is the main component of the healing tissue. This is why the frequency of application of LLLT was effective, as it was carried out during the cellular phase when the number of osteoblasts was increasing. Later, the higher number of cells resulted in a larger deposition of bone matrix, which later incorporated CHA, characterizing maturation of the bone around the implant.[3] The results of the present investigation evidenced a reduction of about 30% in the healing time of the bone, as the concentration of CHA at day 30 is similar to the observed at day 45 in both irradiated and control samples. Normal healing of bone defects and the placement of implants on rabbits is recommended at 42 days after surgery.[26] It is possible to reduce the loading time of implants in the mandible of humans from 4 to approximately 2 months and 24 days, and in the maxillae, from 6 to 4 months and 6 days. Session and treatment doses were also effective as previously described by our team[30,31] and by other groups using IR laser radiation in bone healing.[11,32,33] It is concluded that the use of LLLT was effective in improving bone healing as a result of the increasing deposition of CHA measured by Raman spectroscopy.

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