„VICTOR BABEȘ" UNIVERSITY OF MEDICINE
AND PHARMACY TIMIȘOARA
FACULTY OF DENTAL MEDICINE
DEPARTMENT II

LUCA RUXANDRA ELENA

DOCTORAL THESIS

STUDY ON THE INFLUENCE OF PHOTOBIOMODULATION THERAPY ON THE BIOLOGICAL MECHANISM OF GUIDED BONE REGENERATION

- S U M M A R Y -

Scientific coordinator
PROF. DR. CARMEN TODEA

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INTRODUCTION

Alveolar ridge resorption subsequent to the loss of tooth is a common phenomenon. Immediately after the extraction of a tooth, alveolar ridge width and height decrease rapidly, with a loss of about 40% to 60% of their values in the first three years after extraction. Then, this percentage drops by 0.25% to 0.5% per year. The concept of bone regeneration has emerged as a natural consequence of the need for oral rehabilitation using implant supported restorations in cases of partially or totally edentulous patients, who do not benefit from a sufficient supply of bone. Oral rehabilitation of partial or total edentulous patients with dental implants has become a routine treatment in recent decades, with long-term sustainable results.

The use of laser therapy in the field of implantology has a wide applicability, due to the positive effect that laser radiation exerts on tissue repair processes. The role of photobiomodulation in the processes of healing, repair and bone regeneration was based on previous studies, which showed that fibroblasts irradiated by photobiomodulation produced a higher amount of collagen. On the same reasoning, it went further, considering that laser-irradiated osteoblasts will be stimulated in order to form the osteoid matrix. Due to the positive effects on bone metabolism, observed especially in the case of fracture consolidation, the use of low energy therapeutic laser (hereinafter referred to as LLLT or photobiomodulation) has been encouraged in clinical practice. Effects related to biomodulation include increased vascularity, increased osteoblastic activity, organization of collagen fibers, and changes in mitochondrial and intracellular levels of adenosine triphosphate. It is a non-invasive method of stimulating osteogenesis and accelerating the healing of bone defects[1-4].

OBJECTIVES OF THE STUDY

The **first objective** is to develop an experimental model that would allow the reproduction of the study on a sufficient number of subjects, so that the results could be evaluated statistically. The present study aims to analyze defects created in the calvarial bone of the studied animals, this being a versatile model, which allows the evaluation of biomaterials and bone tissue engineering in a reproducible orthopedic site, which is free of mechanical loads.

The **second objective** refers to the knowledge of the role and benefits of laser therapy in bone augmentation and regeneration procedures. To achieve the desired effects, the operator must have a solid knowledge regarding the use of laser therapy, which includes: radiation doses, its application points, the specificity of the wavelength indicated in post-surgical treatments, as incorrect use of laser radiation can cause uncontrolled effects. The effects of biomodulation, in association with biomaterials in bone defect repair processes, need further study, as different responses from the host organism have been found.

A **third objective** and particular aspect of this research is to stimulate collaboration with international centers specialized in this field, which are interested to make their contributions in these areas of medical interest. The publication of multicenter studies have a decisive role in the effective evaluation and implementation of these methods of treatment. Achieving this goal is intended to be done by integrating in this study several technologies with specificity and applicability in our field of interest, namely micro-CT using Synchorotron radiation, optical coherence tomography, cone beam computed tomography and the gold standard regarding the qualitative evaluation of bone repair - the histological analysis. In this way, we aim to evaluate the process of bone repair in its complexity, not only in terms of quantity or bone microarchitecture, but also in terms of quality, composition and degree of mineralization, to obtain an overview of the results obtained by the proposed technique.

The first part of the thesis presents a series of data from the literature, which represents **the current state of knowledge** in the field of interest and follows several research directions: bone physiology and the biological mechanism of bone healing, prevention of alveolar resorption and the concept of guided bone regeneration, bone replacement materials and membranes used in guided bone regeneration techniques, methods for assessing the quantity and quality of bone substrate, the mechanism, role and benefits of photobiomodulation in bone augmentation and regeneration procedures, general information on photobiomodulation in the field of dentistry, the dose of laser radiation, the mechanism of action of laser photobiomodulation, the effects of laser radiation on bone tissue.

The second part presents in detail the materials, methods and results obtained through **personal contribution to the research**.

SUMMARY OF PERSONAL CONTRIBUTIONS

I. ESTABLISHING THE EXPERIMENTAL MODEL, THE SURGICAL PHASE AND PHOTOBIOMODULATION TREATMENT

In the first part of the study, we decided to use the Wistar breed. We established the investigated region to be the calvary (parietal bone), due to the advantages previously exposed, and the size of the critical defect was set at 5 mm. The ethical approval for the preparation of bone samples was obtained from the International Animal Care and Use Committee on Ethics at the Experimental Research Institute of the Victor Babeş University of Medicine and Pharmacy of Timisoara, Romania and 24 Wistar rats with an average weight of 287 g (range 247-312 g) were randomly distributed into three study groups, each of them having 3 different time periods, during which the process of bone healing was observed, as follows:

- the *negative control group (NC)*, in which 5 mm critical defects are created in the calvarial region, after which the animals are sutured and allowed to heal spontaneously, for a period of 14, 21 and 30 days, respectively
- the *positive control group (PC)* in which 5 mm critical defects are created in the calvaria region, the defects are filled with xenograft and covered with collagen membrane, after which the animals are sutured and allowed to heal for a period of 14 , 21, respectively 30 days
- the *study group (+ LLLT)*, in which 5 mm critical defects are created in the calvaria region, the addition of xenograft and collagen membrane defects are performed and photobiomodulation treatment is applied immediately postoperatively and subsequently, on alternate days, for a period of 14, 21 and 30 days, respectively.

The study group is exposed to low-energy laser radiation using Gallium-Aluminum-Arsenic equipment (IRRADIA Mid-Laser Stockholm, Sweden), with a working length of 808 nm, following a protocol established by our research team: 2J / cm^2 in the first, second and third day after surgery, then alternately (1 day irradiation, 1 day break) in the next 14, 21, and 30 days, respectively, depending on the study group to which the animal subject belongs. The photobiomodulation protocol involved the use of the plastic guide made intraoperatively, to replicate the same precise position in the 5 energy delivery points: one point centrally located, in the middle of the defect and four points located diametrically opposite, on the edges of the 5 mm defect.

II. STUDY OF THE SAMPLES USING MASTER SLAVE OCT

SCANNING OF HARVESTED SAMPLES

In order to study the formation of new bone tissue inside the defect - with or without the grafting material - each of the four quadrants of the defects is imagined separately, using the internally developed CMS / SS OCT system. The interface with the edges of the defect, respectively with the native bone is of special interest, in order to study the process of bone neoformation, which, most often, is initiated from the periphery of the bone defect.

To use the CMS / SS OCT multimodal system in the quantitative assessment of bone healing, the different types of bone tissue must be perceived distinctly on each *en-face* image obtained during the imaging process. Brightness thresholds should therefore be allocated to each type of bone tissue, taking into account the differences in mineralization between them. For this, a software was developed to establish the brightness thresholds and to generate, based on them, the percentage volumes of the investigated tissue types.

RESULTS AND DISCUSSIONS

The CMS software used for 3D / volumetric reconstruction of the sample produces 600 images over an axial range of 1.6 mm, for all four quadrants. The formation of new bone tissue is compared between the three groups, in three moments of time: (I) t1 = 14 days, (II) t2 = 21 days and (III) t3 = 30 days. Thus, it results the display of 12 images obtained from the surface of 2.8 × 2.8 mm^2 of quadrant - for each of the samples considered. The investigations as well as the statistics on the data obtained included all four quadrants and thus the entire area of interest, including the periphery of the defect, as a way to reduce errors with respect to the entire curvature of the sample and the amount of native bone included in the sample, in certain images.

The statistical analysis highlights the information obtained on the healing curve of bone tissue: by performing a comparison between the three study groups, the results showed significant differences in the healing process, depending on the time of healing and the treatment applied. Differences of statistical significance were found between the two control groups, positive B and negative A. The percentage of newly formed bone was higher for the healing period of 30 days than for 21 or 14 days, both for negative control, Group A and for positive control, Group B. The most important variation was found by making a comparison between groups B and C, thus evaluating the effect of photobiomodulation on the healing process of bone tissue, in case of application of guided tissue regeneration techniques. Significant differences were found during the statistical analysis for the 21-day healing period, where it was observed that photobiomodulation had the greatest effect, by inducing the formation of the largest amount of new bone. The results obtained with OCT were confirmed when performing histological examination of the samples.

The results of the present study suggest that the positive effects of photobiomodulation on bone repair processes depend on time, having a greater impact during the early stages of healing. This conclusion is supported by the results of other research groups, which used methods of investigation other than OCT[5-7]. Qualitative histological analysis and histometric analysis showed that photobiomodulation can improve the bone formation process in bone defects filled or not with bovine bone graft, but without accelerating the resorption of particles of this material inside the bone defect[8].

CONCLUSIONS

A quantitative analysis of the healing process of calvarial bone defects of Witsar rats was performed using optical coherence tomography. To the best of our knowledge, this is the first time when OCT has been used as a tool to evaluate the effects of laser photobiomodulation on bone regeneration. Despite the inherent limitations of the method, the results obtained and the statistics performed using OCT were in agreement with previous studies that addressed bone healing using other methods, including the gold standard of qualitative assessment of bone tissue, the histological examination.

As such, I believe that this research represents a breakthrough in the *en-face* imaging as a method of assessing bone regeneration. The major advantage of the method is that it can be applied for *in vivo* evaluations, using hand held samples for laser scanning in the oral cavity[9].

III. HISTOLOGICAL STUDY OF SAMPLES

HISTOLOGICAL EXAMINATION OF THE SAMPLES

Although invasive and sensitive as a working technique, the gold standard in terms of tissue healing processes remains the histological examination, which is why the experimental study conducted includes the realization and interpretation of histological sections. A number of biological parameters must be evaluated in the repair of bone defects, for example, the amount of newly formed bone, the remains of biomaterials, the cells present, the vascularity and porosity of the biomaterial, the inflammatory or foreign body reaction.

In the present study, the samples obtained from the animal experiment were fixed in 10% formalin solution, followed by a moderate descaling agent. The paraffin blocks resulting from the processing were used to make additional sections (4 μm thick, Thermo Scientific ™ HM 355S Automatic Microtome, USA), which were stained with hematoxylin and eosin (HE) and further examined at a Leica DM750 microscope (Leica Microsystems, Wetzlar, Germany).

HISTOLOGICAL ANALYSIS RESULTS

The histological examination revealed interesting aspects, different for the groups NC (negative control), PC (positive control) and + LLLT (study group, which underwent photobiomodulation treatment), depending on the healing period.

On the examined fragments harvested on the 14th day, extensive areas of necrosis and blood extravasation were identified in the group subjected to spontaneous healing. The other two groups (PC and + LLLT) showed a homogeneous eosinophilic material, a "foreign body" with the formation of focal granuloma. The fragments of the + LLLT group showed well-represented fibrous connective tissue (young) and low inflammatory infiltrate, compared to the rest of the groups.

Fragments harvested 21 days after surgery showed a reduction in the inflammatory infiltrate and foreign body granulomas. They were located mainly at the periphery, the fibrous connective tissue comprising the eosinophilic material. The NC group revealed fibrotic connective tissue that included optically opaque areas and more inflammatory infiltrate. The + LLLT group presents newly formed bone tissue, with areas rich in osteoblasts.

After 30 days of healing, it was shown that fibrous connective tissue incorporates the homogeneous eosinophilic material and newly formed bone lamellae, osteoblasts being in large numbers around the bone lamellae, and the inflammatory process was present, as evidenced by giant multinuclear cells.

DISCUSSIONS AND CONCLUSIONS

In our experimental animal study, histological examination revealed significant differences regarding the presence of the inflammatory infiltrate in different study groups: at 14 days, the small amount of inflammatory infiltrate in the + LLLT group allows the organization of young connective tissue, which acts as a precursor of newly formed bone tissue. When analyzing the healing period of 21 days, the reduction of the inflammatory process is more evident in the PC and + LLLT groups. At the same time, as the bone tissue is formed, the + LLLT group presents areas rich in osteoblasts. As the healing period increases, the differences between the analyzed groups in terms of inflammation are reduced, but giant multinuclear cells can still be detected. In a similar study, histological evaluation showed a statistically significant increase in bone formation in the +LLLT group compared to the control group ($p < 0.05$). In addition, inflammation was significantly reduced in the +LLLT group compared to the control group[10].

In conclusion, it appears that the maximum effects of the photobiomodulation occur in the early stages of bone healing, when a smaller amount of inflammatory infiltrate is associated with increased formation of young bone tissue. Although of significant importance, histological evaluation is invasive, which does not allow further examinations of the same samples.

IV. STUDY OF THE SAMPLES USING SYNCHROTRON MICROTOMOGRAPHY

SCANNING OF THE HARVESTED SAMPLES AND QUANTITATIVE EVALUATION OF THE NEWLY FORMED BONE

X-ray microtomography (micro-CT) of the samples was performed at SYRMEP, within the ELETTRA synchrotron research base (Basovizza (TS), Italy). Complete tomographic reconstruction was performed using the open source software SYRMEP Tomo Project (STP)[11]. Subsequently, VG Studio MAX 1.2 software (Volume Graphics, Heidelberg, Germany) was used to generate 3D images, where the gray levels are proportional to the mass density ρ. The Scatter HQ algorithm with an overlap factor of 5.0 was used to imagine 2D sections and 3D reconstructions. Different peaks in the gray level scale represent different phases within the samples; the volume of each phase was obtained by multiplying the volume of a voxel (~730 μm^3) by the number of voxels underlying the peak associated with the relevant phase. A manually set threshold was applied to the histograms to separate the bone under remodeling from the mature bone, and the mature bone from the scaffold phase.

Moreover, the refractive index n signal, linearly proportional to the mass density, was exploited to compute the relative bone mass density distribution (MDDr) of each sample. As recently done in other studies[12], the MDDr parameters were calculated with strict reference to the mineralized bone portion of the histograms, with the intensities normalized, for each sample, by the area under the curve. As the samples are comparable in size and composition, the relative differences in the density distribution between them can be evaluated.

RESULTS AND DISCUSSIONS

Two aspects are evident in the 3D reconstructions of the represented samples: first, the newly-formed bone mainly forms on the borders and not in the center of the defect, and this occurs not only in the NC group, but also in the PC and +LLLT groups (i.e., where the xenograft is present); secondly, with the exception of the +LLLT group, an increase in the volume of newly-formed bone is evident in the period between the 14^{th} and the 21^{st} day after surgery. Moreover, even if the massive presence of the biomaterial prevents a fully reliable evaluation, it appears that after 14 days from surgery, the thickness of newly-formed bone on the defect borders is higher in the +LLLT group than in the PC group. This event is not as evident for longer amounts of time.

The first stage of the study focused on investigating bone microarchitecture and evaluating the volume percentages (vol.%) of different mineralized phases (remodeling bone tissue, mature bone tissue and biomaterial), in relation to the total mineralized bone volume. The percentages of bone tissue being remodeled relative to the amount of mature bone tissue increased over time. This trend was observed in all groups, except for the laser-treated (+ LLLT), where it was found that 14 days after surgery, the newly formed bone tissue is already in greater quantity.

The second step of study was focused on the investigation of the relative bone mineral density distribution (MDDr), i.e., on the evaluation of the calcium concentration and distribution (weight%) in the different groups of study. Thus, the same sub-volumes previously investigated for the calculation of volume percentages were also investigated for the MDDr mapping. In the NC and PC groups, the peak and the mean values followed a similar trend, which decreases over time. This was not the case for the +LLLT group, where a specific trend was not present. The opposite behavior was detected when considering the FWHM values: a specific trend in time was not present in the NC and PC groups, but in the

+LLLT group, where there was a clear decreasing trend over time, with special reference from 14 to 21 days after surgery. Finally, the high value decreased over time in all the groups. Interestingly, after 14 days, the opposite trend was detected considering the FWHM values, while the peak, the mean and the low values were at their maximum in the NC group and minimum in the +LLLT group.

For longer time-points than 14 days (i.e., 21 and 30 days from surgery), when compared with the NC samples (in which the cavities were left empty for spontaneous healing), defects filled with xenografts, i.e., both the PC and the +LLLT samples, presented lower or similar volume percentages of bone under remodeling (i.e., less newly formed bone). The evidence that defects left for spontaneous healing presented already after three weeks of healing (21 days group) more newly-formed bone than cavities filled with xenograft could most likely be caused by a certain delay of the healing process in the presence of biomaterials. We observed this delay in several of our previous studies; specifically, we observed that regenerative kinetics in *in vitro* cultures on different biomaterials showed that the bioresorption of the scaffold is more accentuated up to the second week of culture, while bone regeneration is delayed in time, most likely because cells growing onto the scaffold took longer time to adhere and then to begin proliferating[13-15]. Moreover, in the present study, the biomaterial may have exerted a shielding action in respect to photobiomodulation effects on cells, inhibiting the same regenerative action of the laser treatment.

However, in our study, the quantitative volumetric analysis of bone under remodeling at the three time-points (14, 21 and 30 days from surgery) showed better healing when photobiomodulation was applied on the grafted defect than in cases where the grafted defect did not receive laser treatment. This effect was particularly evident for the shortest considered period of time, i.e., 14 days after surgery. Thus, these observations obtained using our innovative protocol of analysis highlight the positive effects of laser therapy on bone regeneration process, which increase the quantity of newly formed bone. It also suggests possible interactions with the grafting materials that could influence our future experimental follow-ups.

CONCLUSIONS

In our demonstrative study[16], micro-CT allowed us to achieve new and relevant information, although a limited number of rats was included in the study. The power of our protocol lies in the 3D nature of micro-CT analysis, based on the stacking of 1000 successive 2D sections (each with a thickness of about 9 μm), mapping the entire sample. This is of paramount importance, allowing us to minimize the number of rat sacrifices, in full respect to ethical international rules. Our previous studies, using the same method of evaluation, also showed the capacity of micro-CT technique to play a fundamental role in the advanced characterization of laser-treated sites[17]. Moreover, good agreement between OCT and micro-CT analyses was found in our previous studies[18,19].

V. RETROSPECTIVE STUDY OF COMPUTED TOMOGRAPHY WITH CONE BEAM IN PATIENTS TREATED WITH PHOTOBIOMODULATION

INTRODUCTION AND AIM OF THE STUDY

Bone density therefore has an impact on the success of dental implants, a low bone density increasing the risk of failure. In addition, poor bone quality and quantity are reported as risk factors associated with excessive resorption and impairment of the healing process after implant placement[20-23]. In order to assess the qualitative and volumetric characteristics of the intraoral bone tissue, it was proposed to use cone beam computed tomography (CBCT). CBCT offers several advantages over MSCT, such as increased accessibility, lower costs, greater patient comfort, and lower irradiation. Numerous studies have shown a high correlation between CT and CBCT values in terms of shades of gray; therefore, it is suggested that voxel values of CBCT may be used, within certain limits, to estimate bone mineral density[24-28].

The aim of the study is therefore to obtain a quantitative analysis of bone mineral density in patients who underwent photobiomodulation treatments, performing measurements which are specific to computed tomography with cone beam, before and after the application of laser treatment to each case. The objectives of the study include the analysis of the effects of photobiomodulation treatment on the human jaw bones and the evaluation of the applicability and accuracy of computed tomography with cone beam in assessing bone mineral density.

MATERIAL AND METHOD

The study included retrospective analysis of maxillary cone beam computed tomography of 14 patients, 8 women and 6 men, aged between 24 and 74 years, without associated pathology or medication known to affect bone metabolism or its qualitative and quantitative properties. All patients followed the same laser treatment protocol after completion of the sugical procedure: immediately after performing the suture, the intraoral probe of the Gallium-Aluminum – Arsenide laser (GaAIAs) (MID-laser; Serial no 8110131-4) of 808nm, 450 mW was applied, in pulsed regime, administering an energy of 2 J in 3 points corresponding to each inserted dental implant: mesial, distal and apical, cumulating 6J / implant. The treatment sessions were performed immediately postoperatively and every alternative day, for 2 weeks (a total of 8 sessions), in compliance with all laser safety precautions (both the patient and the medical team wore appropriate goggles for the used wavelength).

All images obtained were recorded using the digital imaging and medical communications (DICOM) format, using the same image acquisition parameters (voltage, current intensity, size voxel and visual field). Romexis 4.5.1.R software (Planmeca, Finland) was used to analyze the CT number, expressed in HU. For each patient, bone density was analyzed before and after the application of photobiomodulation treatment, in the same areas of interest, with the same area size for each patient. To locate the target sites and record the average CT number, as well as the standard deviation, we used the Romexis 4.5.1.R software (Planmeca, Finland). Each voxel in this volume is characterized by a CT number, expressed in HU. The software displays the average value of the CT numbers, their standard deviation, as well as the range of HU values (the lowest and highest HU values encountered in the analyzed volume).

All measurements were analyzed statistically, the results being presented in the dedicated section.

RESULTS

The comparative evolution of bone mineral density, quantified by HU, at the level of the maxillary cortical bone, as well as of the medullar bone, before and after photobiomodulation therapy, for all patients included in the study, are presented in detail. The statistical analysis applied to the measured values (ANOVA test) followed some comparisons:

- In both females and males, the differences between the averages of Hounsfield units measured at the level of the buccal cortical bone, before and after treatment, comparatively, show statistically significant differences (Anova test, Tukey test)
- the averages of the Hounsfield units measured at the level of the maxillary medullar bone marrow, before and after treatment, comparatively, show statistically significant differences, both for women and men (Anova test, Tukey test)
- the comparison of the anterior and posterior areas in terms of the averages of the Hounsfield units measured at the level of the cortical and medullary maxillary bone, respectively, does not show statistically significant differences.

- regardless of the patient's sex, both in the buccal cortical bone and in the maxillary trabecular bone, there are statistically significant differences before and after treatment.

DISCUSSIONS

In the study presented in this thesis, the results obtained demonstrate that CBCT is a useful and appropriate tool for measuring bone mineral density in the human jaw bones. Moreover, in terms of the ability to detect statistically significant differences in patients undergoing laser photobiomodulation treatment after oral surgery, laser therapy was shown to increase bone density in both cortical and medullar bone, independent of the patient's sex. This positive effect was observed and quantified both in the anterior maxillary bone and in the posterior areas, the demarcation line between them being considered a tangent line to the distal face of the canines on each side. The quantification of the photobiomodulation effect on bone mineral density using cone beam computed tomography, allowed the formulation of clinical conclusions, in accordance with the experimental ones, obtained in the studies we performed previously.[16,18]

CONCLUSION

According to studies available to date, the indication of cone beam computed tomography as the examination of choice for the determination of bone tissue mineral density, especially when the values obtained are compared with the predetermined standard values, has long been discussed. The present study, based on a comparison between two different moments in time, of the same patients, aims to quantify changes in bone tissue depending on time and treatment, but subject to the same sources of error in the CBCT equipment used. In this way, the usefulness of cone beam computed tomography is obvious, proving to be a valuable predictive factor in assessing bone density. The study demonstrated results with statistical significance, in terms of improving bone mineral density in the case of patients undergoing laser photobiomodulation treatment.

VI. FINAL CONCLUSIONS AND PERSONAL CONTRIBUTIONS

Starting from the premises already known and investigated in the literature, the present research aimes at a qualitative and quantitative analysis of the bone healing process and of photobiomodulated bone regeneration, using optical coherence tomography, computerized microtomography, histological study of samples and, not least, the measurement of bone density using cone beam computed tomography. Such studies are essential to evaluate the effectiveness of different techniques that can be used in bone regeneration, taking into account in particular the complexity of the healing process and the large number of factors that influence it.

Multimodal CMS / SS OCT was applied to render images and analyze different groups considered in the evaluation of the healing and bone regeneration process, the results obtained can be summarized as follows: the largest amount of newly formed bone tissue is found in the study group C, followed by group B and later by group A. Analyzing the evolution of the amount of newly formed bone over time, the most significant difference was highlighted after 21 days, so after about two thirds of the total time interval analyzed. After more time considered, the amounts of bone tend to approach, tending to fill the available defect. From current knowledge, this is the first time that OCT has been used as a tool to evaluate the effects of laser photobiomodulation on bone regeneration. The major advantage of OCT is that it can be applied for *in vivo* evaluations, using manual scanning samples, in the oral cavity[9]. Therefore, I consider that this paper represents a breakthrough in the *en-face* imaging as a method of assessing bone regeneration.

Computed microtomography complemented the previously acquired knowledge, demonstrating that photobiomodulation therapy is effective in short periods of time; photobiomodulation doses given later than 2-3 weeks after surgery do not appear to be very effective. In the conducted study, quantitative volumetric analysis of the regenerated bone at three different time points showed better healing when photobiomodulation was applied to the grafted defect than in cases where the grafted defect did not receive laser treatment. This effect was particularly evident for the shortest period of time considered, i.e. 14 days after surgery.Thus, these observations obtained using the innovative analysis protocol presented above, highlight the positive effects of photobiomodulation on the process of bone regeneration, with the consequent increase in the amount of newly formed bone. It also suggests possible interactions with the biomaterials used, which could influence our future experimental studies. I hypothesized a possible xenograft shielding action in terms of laser action on cells and I intend to verify this assumption through future studies. This effect can be combined with a delay in bone regeneration in the presence of biomaterials, as already documented in previous studies. In the study presented in the thesis[18], micro-CT led to obtaining new and relevant information about a limited number of animals, which were included in the study. Our previous studies using the same evaluation method have also shown the ability of the micro-CT technique to play a fundamental role in the advanced characterization of laser-treated sites[17]. Moreover, in previous studies, a concordance was found between OCT and micro-CT analyzes[18,19, 29,30].

According to the studies available to date, the indication of cone beam computed tomography as the examination of choice for the determination of bone tissue mineral density, especially when the values obtained are compared with the predetermined standard values, has been challenged. The present study, based on a comparison between two different moments in time, of the same patients, aims to quantify changes in bone tissue depending on the time and treatment performed, but subject to the same sources of error in terms of method limitations. Moreover, in terms of the ability to detect statistically significant differences in patients undergoing laser photobiomodulation treatment after oral surgery, laser therapy was shown to increase bone density in both cortical bone and medullary bone, independent of the patient's sex. This positive effect was observed and quantified both in the anterior maxillary bone and in the posterior areas. The quantification of the effect of photobiomodulation on the bone mineral density using cone beam computed tomography allowed the formulation of some clinical conclusions, in accordance with the experimental ones, obtained in the studies we performed previously[16,18]. In this way, the usefulness of cone beam computed tomography is obvious, proving to be a valuable predictive factor in assessing bone density.

In general, the interaction between laser radiation and different tissue types *in vivo* remains a major concern when establishing clinical protocols. Although numerous studies on the effects of photobiomodulation have been performed, their comparison is difficult due to different biomaterials, variations in laser energy, dose and duration. I consider that the present study, correlating the experimental results with the retrospective clinical ones, brings valuable information in areas of current interest, such as bone regeneration and photobiomodulation in the field of dentistry, while clarifying the usefulness of various methods of scientific and paraclinical investigation of bone healing.

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