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

GIANFRANCO SEMEZ

PhD SUMMARY

MULTIDISCIPLINARY APPROACH TO OSTEO-INTEGRATED POST-EXTRACTIVE IMPLANT WITH IMMEDIATE OCCLUSAL LOADING: EXPERIMENTAL AND ANIMAL MODELS

Scientific Coordinator

PROF. UNIV. DR. TODEA DARINCA CARMEN MARILENA

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REZUMAT

The replacement of missing teeth by means of endo-osseous titanium implants has been proven to be a successful treatment modality for both completely and partially edentulous patients. This concept is mainly based on the biologic phenomenon of osseointegration, which has been characterized as a direct structural and functional connection between organized, living bone and the surface of a load-bearing implant. A prerequisite for a successful osseointegration, is the establishment of a direct bone-to-implant contact without the interposition of non-bone or connective tissue. This might be compromised by an exposure of bone tissue to heat formation during conventional drilling procedures, as the threshold level for osteocyte damage has been reported to be around 47° C, only about 10°C above the body temperature, and by the presence of a smear layer around the channel walls. To minimize the risk of temperature increase in the adjacent alveolar bone, an intermittent drilling technique using sharp burs, executed in a sequence of preparation steps under sufficient irrigation with sterile saline solution, has been recommended. Several in vitro studies have also shown that temperatures may increase when drills are used multiple times.

Bone tissue is a specialized form of connective tissue, characterized by the mineralization of the extracellular matrix which confers on the tissue a notable hardness and resistance. Notwithstanding its hardness and resistance to pressure and traction, the bone is very light; this association between maximum resistance and minimum weight is one of the most important attributes of the bone and it is due to its extraordinary internal organization. The most salient characteristic of adult bone tissue is its lamellate structure; the tissue is organized in thin layers varying in thickness from 3 to 7 µm grouped in parallel strata and variously laid out. Among all mechanically functioning tissues, bone tissue is the most highly differentiated. In its ontogenetic development it is preceded by connective tissue or else by connective tissue and subsequently by cartilage.

Related to the stability concept, "primary stability is considered a fundamental prerequisite for promoting the carrying out of osseointegration" (Brånemark et al. 1977, Adell et al. 1981) and it is also the most important factor in immediate loading. It can be defined as an initial fixed location of the implant in the osseous site strong enough to bear the dislocating forces that operate on the implant itself. If the operating forces, even of minimal intensity, are such as to cause mobility in the implant during the period of healing there can be observed an alteration in the process of osseous formation which can lead to the failure of the implant. Initial stability is determined by the ability of the receiving bone to oppose the micro-movement of the implant during the period of healing.

Peri-implant re-absorption is considered physiological when the loss of marginal bone is 1,5mm vertically before the occlusal load is less than 0,2 mm annually. It is supposed that there are various factors that influence this physiological phenomenon and many of them are linked to the characteristics of current implants.

In recent years, the use of laser radiation has also been expected to serve as an alternative or adjunctive treatment for bone tissue ablation due to the vaporization of the tissues and absence of the smear layer. Close attention has been paid to the clinical applicability of the Er:YAG laser with a wavelength of 2940nm in the near infrared spectrum because this wavelength is absorbed by endogenous and exogenous water. This laser system provides a capability to ablate bone effectively without producing major thermal side effects to adjacent tissues. In particular, the results from a recent study analyzing the ultrastructure of rat parietal bone surface demonstrated that Er:YAG laser irradiation resulted in a very thin changed layer of approximately 30 µm thickness, which consisted of a superficial, greatly altered layer and a deep, less affected layer. Lewandrowski et al. reported that bone healing following Er:YAG laser irradiation in an inferior border defect of the rat mandible appeared to be equivalent or even faster than that following bur drilling. All these data seem to indicate that the evolution of the implant surfaces associated with the use of new devices (ex: laser) may create a structural synergy of action both between OsseoTite™ and NanoTite™ surfaces (or new surfaces under study) to guarantee an elevated bioactivity, in order to obtain an increase of (the) implant performance especially in difficult clinical situations such as poor bone quality and immediate functional occlusal loading. Indeed, preliminary results from experimental studies on rats have indicated that titanium screws are able to osseointegrate in an Er:YAG laser prepared bone defect. Different laser wavelengths currently available on the market allow clinicians to achieve excellent results in oral surgery, mostly due to their specific features. Tissue ablative capability, haemostatic properties, bio-stimulating effect of light on live tissue decontamination and antibacterial action, are only some of the reasons that can lead doctors to choose a laser device instead of a conventional blade. In implant surgery, in particular when there is no need to improve or to preserve the amount of adherent gingiva and a two step implant protocol was chosen, the approach to the uncovering of the fixture can consist of an over implant mucous operculum. In this way, a laser integrated approach can provide many advantages, such as the reduction or the absence of anesthetic use, (the) reduced or absent bleeding, the excellent healing of tissue during pre-prosthetic conditioning.

The LASER is a device that generates or amplifies light. In this case by light we must understand a gamma of single wavelengths that go from distant infrared through the visible spectrum to deep ultraviolet. The radiation rays that lasers emit or amplify have peculiar physical characteristics such as monochromicity or spectral purity (the laser possesses only one vibration frequency, hence only one color characteristic of the active means that has produced it), coherence or intensity (all the photons vibrate in phase concordance) and unidirectionality (the radiation leaves the laser device in a certain direction and is diffused at a definite angle of divergence in the order of milliradiants. This property is also called collimation.

Lasers can be classified based on:

- Active medium (Mezzo attivo) (solid, liquid, gas…)
- Spectral wavelength (visibile, IR, UV)
- Pumping system (optical , electrical…)
- Number of energy levels
- Risks connected (ANSI)

A more immediate classification of the lasers used in dentistry, for the operator, could be that linked to the depth of penetration of the rays interacting with the tissue.

The propagation of the laser-beam on target tissue is described as having the fundamental optical properties; these are the reflection, the refraction, the absorption, the diffusion and the transmission.

About 5% of the incident ray is reflected because the refraction index differs from the external medium and the tissue. 95% spreads in the tissue where it is absorbed and diffused. There are several substances in tissue that interact; absorbing the laser beam and its effect varies according to wavelength variations. Water plays a fundamental role in that it represents the main chromophore in the spectral region of infrared. In the optical bands of the visible and of infrared the radiation diffusion is noticeable and this is due to the numerous cell structures in the tissue. As a result, three other typologies of luminous radiation propagation in tissue can be found which depend on the wavelength of laser radiation and on the characteristics of the tissue used.

Related to the indications and contraindications of the lasers in implantology, high esthetic demands with conservation of the integrity of soft tissue and, probably, of hard tissue too, need for a necessity of reduction of the number of surgical sessions and the overall duration of treatment. The advantages are absence of discomfort and vibration, less chance of contamination of the surgical site, reduced risk of trauma to adjacent tissue, minor thermal injuries, bio - modulation, absence of charring so an improvement in healing and less postoperative discomfort.

The impossibility of obtaining good mechanical stability of the implant due to extensive bone loss of the socket, the presence of visible endodontic x-ray lesions and\or presence of acute infections (endo or perio), gingival lesions impossible to treat with regenerative procedures in the same field, before the placement of a post-extractive implant, the anatomic features of the tooth that necessitate a traumatic extraction by osteotomy, the tooth position in arch or socket morphology which makes the site unfavorable for an immediate post-extractive implant are absolute contraindications .

The disadvantages of the laser employ are the more expensive device, the specific preparation of the operator, the impossibility of correctly repositioning the scanner, the difficulty in measuring the length of preparation during surgery, the impossibility of standardizing the length on the bases of the type of bone linked to greater blood affluence, the cleaning of the glass, the presence of cloud debris, the conical shape of the preparation site.

The presence of the QSP (Quantic Square Pulse) function, recently introduced in Fotona Lightwalker, takes advantage of this new feature.

The positioning of an implant in the bone must be considered as aggressive to the integrity of the organism. No matter how adequate it may be in the planning stage, an implant inserted into the bone always presents a micrometrically empty interface space, the seat of complex biological reactions the evolution of which is conditioned by the primary (mechanical) stability of the implant itself.

A series of factors, identifiable above all in mechanical stimuli, modulate the subsequent cellular differentiation in the osteoblast and fibroblast sense.

The presence of micro-movements in the dental implant can influence differentiation in the fibroblast sense and this could turn out to be preponderant to the extent of insinuating itself into the interface between the bone and the implant.

The primary aim of bone-implant surgery is to achieve the best repair possible of the tissue surrounding the implant both in terms of time and in terms of the quality of the anchorage. It is obvious that an optimal repair of the tissue surrounding an implant depends on limiting the damage to living tissue at the moment of insertion.

SPECIAL PART

CHEMICAL AND MORPHOLOGIC ANALYSIS OF TITANIUM DENTAL IMPLANTS: X-RAY PHOTOEMISSION TECHNIQUES (XPS) AND SCANNING ELECTRON MICROSCOPY (SEM) WITH EDX ANALYSIS

INTRODUCTION

Nowadays the medical market is very rich in different products in the dental implant field, some of them being produced by well-known manufacturers, thus having a higher probability regarding the quality control process.

For a proper osseointegration, some conditions must be fulfilled: biocompatibility, the implant form, macro and microscopic surface of the implant, the loading conditions, the surgical technique and the conditions of the site where the implant is destined.

AIM OF STUDY

Aim of the study is the evaluation of the chemical composition of implant surface and eventual affection of the good and stable osseointegration.

MATERIAL AND METHOD

The morphology and the composition of both samples was done using a Scanning Electronic Microscopy (SEM) coupled to a spectrometer EDX (Energy Dispersive X-ray) and

XPS (X-Ray Photoemission) by Dr. Cinzia Cepek (Istituto Officina dei Materiali Sede di Istituto Trieste, Basovizza). The analysis was performed on two implants group: group 1-BIOMET 3I (sample 1) and group 2-MYIMPLANT (sample 2).

RESULTS AND DISCUSSIONS

The X-ray Photoemission Spectroscopy (XPS) was done using the Analytic section laboratory of the TASC- IOM-CNR (base pressure 10-11mbar). It was utilized a hemispherical energy analyzer of electrons (120mm, PSP) and a conventional non monochromatized Mg x-ray source. All the spectrum is normalized at the photons number and the bond energy was calibrated using a sample of polycrystalline gold. In presence of chargement phenomenons the bond energy was attributed putting the Ti 2p3/2 a 459.1eV.

The composition and morphology of all sample were investigated using a Scanning Electronic Microscopy (SEM) coupled with an EDX spectrometer (Energy Dispersive X-ray). Concentrations obtained by EDX spectroscopy were calculated using the standard software of the device. To better underline the presence of contaminants after the cleaning processes of the implants (aluminum and\or silicate), the results will be presented according to the implant type.

The following obtained values reports the experimental XPS data of all measurements made on the 2 samples. The percentage values of the ions concentration into sample no. 1 are presented. The figures show the behavior of the ions presents on the BIOMET implant surface during XPS treatment.

The same analysis was made on sample no.2, in order to identify the ion concentration percentage (table 5) and the behavior of the ions that are present on the implant surface (MYIMPLANT) during XPS treatment.

The major difference which can be identified between the samples not used by the company's products analyzed is the amount of surface aluminum (in oxidized state, alumina). This appears to be more than an order of magnitude greater (12 times) in the MYIMPLANT sample. We observed that the amount of aluminum compared to titanium in the sample MYIMPLANT (1.2) is significantly higher than the one found in titanium alloys typically used for dental implants (between 0 and about 0.06), while in the sample BIOMET (0.1) approaches and is compatible with these values within the experimental errors.

There are few studies interested in the interaction of these contamination with the process of osseointegration of the implant. No literature studies were found on the biological tissue reaction with this kind of alloy detected by our study. All the studies regarding osseointegration are based on the interaction of living tissues and pure titanium, no other contaminant material is considered on this topic. The implants present in the market can be really different from one to the other, although all of them are classified as Type 5 Titanium.

CONCLUSIONS

The presence of contaminants on the surface of dental implants may lead to a problem with the osseointegration process, that is evaluated in the interaction between tissue and pure titanium. Therefore, we consider that there is a need for further studies regarding the interaction of the contamination materials and the tissues, especially in which regards a better knowledge of different implants alloy compositions, with a better control of these aspects. From the clinician point of view, it is impossible to assess the implant composition. The only data that the clinician has in its hands is the percentage of success obtained in his practice.

PERFORMANCE OF LASER VERSUS TRADITIONAL IMPLANT SITE PREPARATION IN ANIMAL MODEL ACCORDING TO HISTOLOGICAL EXAMINATION

INTRODUCTION

The introduction of endosseous implants in dentistry was the beginning of a new era in dental medicine and the following years have brought many improvements to the implant designs and their insertion techniques. Nowadays, dental implants represent a treatment method, which is well integrated in the daily practice of many dentists. In particular, the use of endosseous titanium implants for replacing missing teeth for both completely and partially edentulous patients has been proven to be a successful treatment modality.

Recently, the use of laser radiation has shown to be an alternative or adjunctive treatment for bone tissue ablation due to the vaporization of the tissues and absence of the smear layer.

Moreover, another important aspect is the implant site preparation is the geometric repeatability of the bone site.

Respect for the surgical sequences for implant site preparation, with calibration cutters growing up to the desired diameter, allow us to achieve optimum positioning of the fixture, allowing an adequate BIC value, thus obtaining a high success predictability as far as concerns the osseointegration process.

Up to now, proper laser devices useful to create a repeatable bone site are missing and so far very few improvements in the development of cutting instruments for implant surfaces are made.

AIM OF THE STUDY

The aim of this preliminary study is to evaluate and compare the use of Er:YAG laser or conventional methods (bur and cutter) on hard and soft tissues using an in vitro model.

MATERIAL AND METHOD

Bovine tongue was used as soft tissue due to the presence of superficial dorsal non-keratinized and ventral mucous non-keratinized tissues, which are histologically similar to non-keratinized gingival tissue. Bovine rib was used as hard tissue since it is very similar to human jaw regarding the bone density and the cortical/marrow ratio. Er:YAG laser, Fotona X-Runner, and circular cutter were used. Holes preparation on the soft tissue was carried out using the Er:YAG (2940 LightWalker AT, Fotona Slovenia – Figure 1) laser with a 5 mm diameter scanner (X-Runner, Fotona, Slovenia) in MAX mode and quantum square pulse (QSP) mode in comparison to a circular scalpel of 5 mm in diameter.

Three samples of each group (soft tissue and hard tissue) were sealed with silicone (Light Body – Aquasil Ultra, Dentsply). Bone samples were collected in duplicate. The bone samples were decalcified [2–4% hydrochloric acid, 4–6% ethylenediaminetetraacetic acid (EDTA) for four days], then processed [Ethanol series (50°–80°–90°–99°–99°,45 minutes each; Xylene three times, 45 minutes each; Paraffin 56°C, three times, 45 minutes each], embedded in paraffin.

RESULTS AND DISCUSSIONS

We observed that on soft tissue the cavity shape performed by laser (QSP mode) is well preserved. The model is characterized by a net and clear border from the top to the bottom between the cavity and the muscular fibrocells, which are typical for the tongue bovine model that was used. They are not evident any kind of alteration of the normal histological structures. This is clearly related to the physical effects of QSP mode.

In soft tissue, we also noticed a thermal effect due to laser used in MAX mode. We noticed, in particular, a continuous collagenization of the surface cavity, as shown in Figure 16, a bluish tissue near the cavity where the normal tissue organization is lost. Photothermal and photochemical activity was highlighted when using MAX mode in tongue muscle. Eosinophilic fibers with nuclei in partial degradation (basophil) and evidence of thermal effect with thermal fiber damage (eosinophilic) at the border between cavity and tissue.

On the contrary, this thermal effect was not observed in those holes performed in QSP mode. This different thermal effect is probably due to a different use of air/water ratio in the MAX and QSP mode.

Moreover, we observed also that the use of silicone for sealing the cavity after preparation is clearly important in maintaining debris and the shape of the cavity itself.

CONCLUSIONS

Our study demonstrated that using this new QSP mode of ER:YAG laser there are not evident any kind of alterations of the normal histological structures in soft and hard tissues, whilst using MAX mode photothermal and photochemical activity was highlighted. Moreover, due to the insufficient number of experimental and clinical studies, the laser still is not considered the first choice in implant bed preparation.

A COMPARATIVE ANALYSIS REGARDING THE OSSEOINTEGRATION OF IMMEDIATE LOADED IMPLANTS USING TWO DIFFERENT IMPLANT SITE PREPARATIONS: ERBIUM:YTTRIUM-ALUMINUM-GARNET LASER VERSUS SURGICAL CONVENTIONAL WAY

INTRODUCTION

The replacement of missing teeth for both, completely and partially edentulous patients, by means of endo-osseous titanium implants is mainly based on the concept of the biologic phenomenon of osseointegration.

A prerequisite condition for a successful osseointegration is represented by the establishment of a direct bone-to-implant contact, without the interposition of non-bone or connective tissue. This might be compromised by an exposure of bone tissue to heat formation during conventional drilling procedures, as the threshold level for osteocyte damage has been reported to be around 47° C and by the presence of a smear layer around the channel walls (1–10).To minimize the risk of temperature increase an intermittent drilling technique using sharp burs under sufficient irrigation has been recommended.

AIM OF THE STUDY

Aim of the study is to evaluate the possibility of preparing the implant site using the scanner prototype and to reduce and/or eliminate the time between the primary stability (mechanical) and the secondary stability (biological) looking at the immediate occlusal loading.

MATERIALS AND METHODS

6 mini-pigs from Ellegaard Gottingen Minipigs 18 months old, 30 kilos weight, with the certification of integrity of dental teeth were used to evaluate post extractive implant positioning with immediate load. Pigs, after sedation and general anesthesia, underwent extraction procedure of four teeth (2 posterior on both sides) with immediate implant positioning.

Experimental procedure can be summarized in different phases: pre-implant, surgical implant positioning and pig sacrifice.

During the implant positioning phase the positioning strength of the implant and the amount of bone adherent to the implant were evaluated.

After the pig sacrifice the bone samples were evaluated for the newly formed bone and for their histological features.

On the left side were positioned 2 implants (T3 cylindric type implant, a commercially tapered prototype in pure titanium (4 mm diameter, 11,5 mm long) with a new surface, Discrete

Crystalline Deposition DCD Biomet 3i) with traditional protocol (1 immediate occlusal loaded and 1 delayed).

On the right side 2 implants were positioned with scanner (X runner) laser protocol Laser Fotona Lightwalker AT MAX Mode (1000 mJ; 20 Hz; air/water ratio 4; diameter 3mm). One of them was immediate occlusal loaded and the other one delayed.

After the re-positioning of the flap using re-absorbable sutures (Vicryl) all the min-pigs were kept on antibiotics (Veterinary protocol) for six days. (Death of two mini-pigs due to salmonella infection and gastroesophageal reflux, Pig number 4 and Pig number 6)

Healing time after this procedure was defined in 45 days.

One mini-pig was sacrificed at day 45 after implant positioning (PIG 1), while the others were sacrificed at days 60 (PIG 2), 90 (PIG 3) and 120 (PIG 5), respectively. Each hemi-mandible was resected en bloc, both containing the positioned implants and immediately placed in 10% fixative, buffered formalin, for histologic preparation and subsequent evaluation.

RESULTS

For those implants positioned in the traditional manner, torque values (measured by a newton metric ratchet) for implant 1 (position 34) and implant 2 (position 37). For those implants positioned by the use of the laser scanner, torques values (measured by a newton metric ratchet) for implant 3 (44) and implant 4 (47) are presented; RFA Ostell (resonance frequency analysis) values for implant 1 (44) are presented.

The histomorphometric analysis was carried out using a light microscope (Nikon Eclipse; Nikon, Tokyo, Japan) connected to a high-resolution video camera; this optical system was associated with a histometry software package with image-capturing capabilities (ImageJ).

For each pig, histological examination is described for all cone bone samples available hereafter.

DISCUSSIONS

An effective comparison between bur implant tunnel preparation and two different healing periods can be performed analyzing PIG 2 and 3. This shows a comparable amount in empty spaces and necrosis of the bone as an increase in woven bone and a reduction in lamellar bone.

Moreover, it is interesting to compare bone composition of implant sites prepared in the same way, at different time stages.

Concerning bone surrounding implants placed with laser technique, samples harvested 120 days after healing (PIG 5) showed more lamellar mature bone and less woven bone than samples harvested 60 days after healing (PIG 2). This could indicate that bone maturation mainly occurs between two and three months of healing.

The same trend occurred in the comparison of the samples harvested after bur implant-site preparation. Percentages of lamellar bone were particularly higher in the sample with 90 days of bone healing (PIG 3) in comparison with the sample healed 60 days (pig 2). Similarly, the amount of woven bone decreased from day 60 to day 90, indicating a bone maturation process.

The choice of the Er:YAG laser as opposed to CO2 is the difference in the increase in temperature. The erbium ray will be absorbed by the hydroxyapatite and by the osseous water. The hydroxyl groups of the osteocyte molecule are the target of the laser ray which brings about an ablation of the apatite.

It has been already demonstrated that the first bony healing phase after implant placement is caused by the surgical trauma. However, during the following weeks it is possible to observe the formation of an immature bone due to the remodeling phase of the bone. In details, after two weeks the presence of osteoid matrix and immature bone are evident, and the mature bone in a direct contact with the implant is the original bone.

After four weeks, there is still a high amount of immature and woven bone but it is possible to see the first lamellae of a much more mature lamellar bone, in direct contact with the implant threads.

After two months from implant placement, we show that the fixtures are well integrated in the surrounding bone with a good corticalization process along the implant surface and many empty spaces are filled up with newly formed mature bone.

This is the moment where it is possible to detect surely if the implant has failed. Our samples harvested 60 days after healing (PIG 2) showed exactly the same corticalization in the implant that was not loaded, since a continuous layer of bone in contact to the implant surface is evident. More in general, those samples analyzed after a healing period of 60 days, showed a delayed bone maturation with still high presence of bone marrow spaces and woven bone, both in the laser group and in the bur group.

After 90 days of healing from implant surgery, the bone is mainly made by newly formed lamellae and a thick layer of continuous bone usually covers the entire fixture. This occurred also in our samples, harvested from pig 3 after three months of healing.

CONCLUSIONS

After four weeks, a high percentage of lamellar mature bone was found in a direct line with the implant surface on the site which was laser-prepared.

Both implants showed bone integration and newly formed mature bone in contact with the titanium threads, mainly in their apical part.

The sample harvested from the laser group showed less empty spaces, probably due to the faster transformation of bone marrow in a more mature bone.

FINAL CONCLUSIONS AND PERSONAL CONTRIBUTION

1. Nowadays the medical market is very rich in different products in the dental implant field, some of them being produced by well-known manufacturers, thus having a higher probability regarding the quality control process. Related to the atomic composition of the implants there are not so many studies. There have been conducted previous studies on titanium with a relatively wide range of conditions for the coatings preparation. We can enumerate: different electrical regimes, treatment times and electrolyte compositions.

2. During this research work we manage to demonstrate that contaminants on the surface of the implant can create after- positioning problems. Numerous articles have dealt with the study of the treatment of the implant surface using lasers. This is dictated by the need to research into the innovative peculiarities to be carried out on the implant surface.

3. The information which are giving to the clinicians in case of implant failure in order to analyze the chemical composition of the implant save the position of doctors from the ethical point of view.

4. The insertion of the implant at the cortical bone level determines a compression of the surrounding tissue and above all of the blood vessels which can induce a phenomenon of necrosis of the bone tissue at the bone-implant interface.

5. New strategies of treatment of the surface of implants have shown an increase in the bone conductive properties introducing a parameter such as BIC to evidence such properties.

6. Until today a correlation between BIC and the stability of the implant has not been established. The stability of an implant is established by the anchorage between the implant and cortical and it is further favored by the macro-geometry of the implant rather than by the configuration of its surface. Although surgical implantation can be carried out in a non- traumatic way, a certain amount of the pre-implant bone becomes necrotic because of the vascular interruption in the Havers and Volkmann canals which vascularize the osteons, carrying nutrition and oxygen by diffusion to the osteocytes. The interruption of the vascularization caused by the action of the cutter on the bone leads to necrosis of the osteocytes and consequently to the devitalization of the bone.

7. Overall, our results suggest that silicone sealing is necessary to maintain the shape of the cavity and the debris inside the cavity prepared using all the devices. **Differences in using or not the silicone are significant**. Indeed, we show that silicone is fundamental in order to measure correctly the shape of the cavity.

The research conducted allowed us to demonstrate:

- the possibility of applying immediate occlusal loading on an *in vivo* animal model;
- how the use of the laser compared to the traditional drill, leading to the presence of a greater share of lamellar bone and a lower necrotic share in the implant site;
- the existence of problems related to the presence of surface contaminants of the implants;
- how the underestimated permanence of residues - debris at the implant site can cause failure.

Our experience in this research has shown that the method used is worthy of scientific interest and needs further investigation for certification or exclusion of its validity.