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Evaluation of bone tissue reaction in laser beamed implants

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ABSTRACT

The purpose of this study was to evaluate alterations and bone tissue response on laser treated implant surfaces (Nd:YAG – 100 W). Sixty grade II titanium (ASTM F67) mini-implants (1.5 mm × 4.0 mm) were installed in femurs of 30 Wistar rats. The animals were divided into two groups: thirty mini-implants were machined elements (Machined Group) and the other thirty had laser beamed surfaces (Laser Group). The animals were subdivided into three groups, according to bone healing periods of 15, 30 and 60 days. The samples were analyzed under light, scanning electron and confocal 3D microscopy as well as by EDS (energy dispersive spectroscopy) and Student's *t* test was used for statistical analyses. Light microscopy results showed new bone trabeculae formation toward laser-treated implants at 15 days' bone repair as well as thin layers of osteoid matrix, indicating high biocompatibility. Similar features were observed in the Machined Group but only after 30 days. Bone/implant contact was better evidenced on laser-treated surfaces compared to that on simply machined implants. The only group that demonstrated change in level of significance was the laser-treated group at the 15-day-healing period ($p < 0.05$). Higher oxygen concentration possibly provides more efficient response of osteoblasts during new bone tissue deposition. Implant treated surfaces altered by laser beaming, their composition, surface topography and surface energy may be the future scene in implant dentistry.

1. Introduction

Titanium is considered as "the material of choice" in the manufacturing of dental implants. When a titanium implant is installed in the maxillary or mandible bone, bone cells identify the surface favoring the migration of defense cells, as well as healing cells, toward the substratum. As a result, there is an almost direct biological link between the bone and the metal element, which indicates its biocompatibility.

Medical and Dental Schools are increasingly intertwined in the development of new biomaterials to be used in repairing of human bodily parts, either damaged by accidents or for pathological reasons. Both areas of Health Studies are involved in the search for new technologies, in order to achieve improved response concerning osteoconduction. Titanium alloy is considered to be a metal with excellent biocompatibility [1] and studies have shown that cells perceive differences on surface characteristics and respond in different ways [2–5].

Bone response following anchorage is influenced by the characteristics on implant surfaces. The extent of contact between titanium and new bone depends on characteristics such as the composition of the alloy, surface topography, surface roughness and surface energy of the implant. Chemical composition and surface topography of titanium implants play an important role in the rate and extent of osseointegration as well as adhesion of bone cells onto titanium surfaces [1]. Changes in the configuration of surfaces can alter the targeting of proteins to be adsorbed, as well as the adhesion of certain cells onto the surface [6]. In order to obtain cellular responses that are favorable in terms of bone deposition, modified titanium surfaces have been used. These include hydroxyapatite coated surfaces, Titanium Plasma Sprayed (TPS) surfaces, sand blasted surfaces, acid etched surfaces [7], anodized surfaces (to affect morphological changes) [8] and biomimetic coatings. These surface modifications help bone repair and accelerate mineralization of the osteoid matrix [9].

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Published data on this subject have suggested that tissue cells migrate more easily because of adequate surface tension, which helps promote such migration of new cells for bone deposition [10]. In early stages, this factor may play an important role in discriminating and in determining which proteins should be adsorbed onto the surface, as well as in promoting or inhibiting the adhesion of certain cells. Surface energy can have an effect in the later stages of bone formation and calcification, influencing the types of cells that initially attach to the implant surface and will differentiate at the interface between the titanium element and the bone tissue.

Surface treatment of titanium implants using high-powered laser beaming is an alternative method to obtain morphological changes. Changes produced by this method concerning physical and chemical properties of the surfaces can help promote and accelerate biomechanical anchoring of implants. In addition, this method does not introduce impurities nor gaseous components onto the surface and, thus, it maintains the metal composition unaltered [11]. However, thermal discharges from the laser pulses could alter the composition and microstructure of the implant surface [12].

Nowadays, laser equipment is widely used in Health Fields. Neodymium Yttrium Aluminum Garnet Laser (Nd:YAG - λ = 1.064 nm), CO_2 (carbon dioxide laser – λ = 9.300 nm, 9.600 nm, 10.300 nm and 10.600 nm) and diode laser treatments with wavelengths of 810 nm and 980 nm have been used in the clinical practices of both Medicine and Dentistry [13].

Laser treatments are used in photocoagulation of blood vessels in treatments of tumors, ocular surgery, vaporization of tissue and kidney stones, removal of skin blemishes or tattoos, in rejuvenating treatments, permanent hair removal, healing of ulcers and bleeding, as well as in the removal of decayed dental tissues [13].

Several advantages are highlighted by the use of laser devices in the oral cavity such as easier control of hemostatic effects and bactericidal effects against periodontal pathogens. However, several potential risks may indicate against high power laser in clinical uses. It was demonstrated in studies of root surface that Nd:YAG laser penetrates the tissue to a depth that can cause damage to the respective underlying layers [14].

The Nd:YAG laser beaming is not suitable for treatments on metal contaminated surfaces (*e.g.* peri-implantitis) because it causes overheating and possible melting of components [12]. The high-intensity Nd:YAG laser was used in studies to modify surfaces of titanium implants [12] where, due to the rapid temperature rise, it generated alterations on their original morphology. Even procedures in light emission energy present considerable risks. A study [15] described the effects of laser treatment with Nd:YAG *in vitro* on the properties of surface plasma spraying on titanium and also hydroxyapatite-coated implants. Effects on the surfaces were examined after laser treatment with 0.3, 2.0 and 3.0 W. Results showed loss of fusion, porosity and surface alterations on the model surfaces of both treatments, even under low power. In another study focused on oxidation, promoted by the impact of laser beams on titanium surfaces, the authors concluded that the oxide layer thickness was modified and that the metal fused due to the amount of emission in one same place [16].

Laser ablation changes the microstructure and increases hardness. Further, it alters roughness, improves corrosion resistance and increases surface oxide thickness [11]. A network of micro craters is formed [12] because of heat stress from laser irradiation [11]. These changes to the surfaces of endo-osseous implants can affect the biological behavior of bone tissue [8] by changing the direction of osteoblasts movement, thus favoring the control of growth direction [2].

Studies about the physical alterations that metallic components undergo when laser is processed in oxygen environments have been the subject of several investigations [11,12,17]. Laser irradiation of metallic surfaces in oxidizing environments (oxygen or air) causes changes in oxide growth at regions upon which the laser beam is incident. Rapid oxidation of the metal surface is considered to occur due to energy release [17]. According to György et al. [12], laser pulses applied to titanium surfaces increase the surface temperature causing the melting of the surface layer, which subsequently solidifies, promoting increased oxidation. The most important effect observed at the target region is the increased oxide layer thickness [11,17], which is due to the release of heat during the oxidation reaction [12].

Rats are favorable, as animal models, due to their small size, low cost and stable metabolism. The animal model used in this study was considered suitable due to the size of the femoral bone according to the mini-implants used, allowing installation at a regular and standardized distance, in proportion to the walls of the femur bone of the animal. Furthermore, selected rats weighing approximately 350 g, in average 9 to 10 weeks old, are considered "adults" when compared to human beings, allowing similar biological comparison to the metabolism of an adult man.

Based on the biological potential of surfaces that have undergone modifications, brought about by various treatments, during the last 10 years, and with the idea of improving and accelerating bone-implant interaction, the purpose of this study was to evaluate the biological response of bone tissue *in vivo*, when using implant surfaces that have been morphologically altered by laser beaming. This response has been compared to that on simply machined surfaces.

The purpose of this study also aimed to a better understanding of the changes that take place in the surface oxide layer with the aid of scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS).

2. Materials and methods

In this study, 60 sterile* mini-implants in grade II titanium (ASTM F67), standard size of 1.5 × 4.0 mm Ø, were installed in the femurs of 30 Wistar rats.

*The evaluation of the endotoxin content in the samples after sterilization by gamma radiation from ^{60}Co was conducted *via* VDMAX (Maximum Verification Dose) method to control the microbial load (bioburden) according to ISO 11137. The result was zero colony forming units (CFU) of microorganisms under irradiation at 25 kGy in 10 units (within same batch of implants used in this research), which also confirmed no presence of endotoxin generator vectors.

Initially, the surfaces were analyzed using different techniques (further discussed below) and, subsequently, surgical procedures were carried out in the animals.

The animals were divided into two groups: In Group I (Machined Implants Group), 30 (thirty) implants with machined surfaces were used while, in Group II (Laser-treated Implants Group), the other 30 (thirty) machined implants had their surface subjected to irradiation with Nd:YAG Laser equipment in order to create and increase surface roughness.

Each of the two groups (Group I and Group II) was randomly divided into 03 (three) distinct sub-groups according the respective bone healing periods of 15, 30 and 60 days, until the removal of the implant samples for analyses.

2.1. Laser surface preparation

Nd:YAG laser (ADITEK) (Cravinhos, SP) was selected for this study because it provides a degree of average power, suitable for engraving and marking surfaces, with limited penetration of less than 1 mm and the ablation process