Surface and Biomechanical Study of Titanium Implants Modified by Laser With and Without Hydroxyapatite Coating, in Rabbits

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Surface and biomechanical analysis of titanium implant surfaces modified by laser beam with and without hydroxyapatite. Titanium implants with 3 different surfaces were inserted into the tibias of 30 rabbits: group I (GI) machined surface (control group), group II irradiated with laser (GII), and group III irradiated with laser and hydroxyapatite coating applied—biomimetic method (GIII). Topographical analysis with scanning electron microscopy was made before surgery in the tibia. These rabbits were distributed into 2 periods of observation: 4 and 8 weeks postsurgery, after which biomechanical analysis (removal torque) was conducted. Statistical analysis used the Student-Newman-Keuls method. Surface showed roughness in GII and GIII. Biomechanical analysis demonstrated values with significant differences in GII and GIII. Titanium implants modified by laser irradiation can increase osseointegration during the initial phase.

Key Words: rabbits, lasers, titanium, dental implants, durapatite

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Introduction

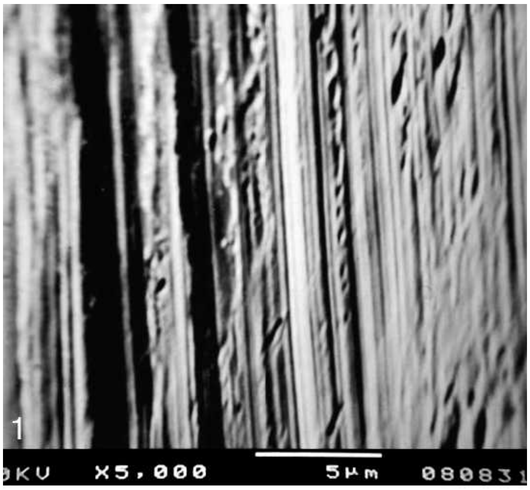
Titanium has proven its suitability as an implant material in surgery over many years. Excellent biocompatibility and corrosion resistance are outstanding features.[1] Osseointegration is the direct structural and functional contact between organized bone and the implant's surface.[2] Biologically, there is no evidence of direct contact between the bone and the implant; what can be determined is a high or low quantity of fibrous tissue at the interface. "Osseointegration is the process by which stable and asymptomatic fixation of an alloplastic material in the bone is obtained and maintained during function."[3] With the objective of improving or even accelerating this process, especially in areas with poor-quality bone, various studies about the modification of the surface of titanium implants have been undertaken in the last few decades, some of which have shown that coating with hydroxyapatite improves bone rigidity and the implant/bone interface.[4] Other examples of methods used to modify the titanium implant surface include titanium and hydroxyapatite plasma spray, blasting with different particles (sand, glass, aluminum oxide), acid etching, anodization, and irradiation with high-intensity laser.[5–9] The oxidation and nitration of a titanium surface with laser ablation is important because it increases the surface area and also because titanium oxide and titanium nitrate are biocompatible.[6–9] Titanium nitration increases the wettability of implants and is considered bioinert by the Food and Drug Administration.[6–9] In 2002, titanium implant surfaces were irradiated, at varying high temperatures, with laser, which changed the original morphologic surface.[10] Laser was used on titanium samples and indicated that adjusting parameters, such as potency and length of exposure, influenced the melted surface.[11] In studies of oxidation by laser on titanium surfaces, Lavisse[12] concluded that the thickness of the melted layer depends on the amount of emission on the same local surface. With regard to the application of hydroxyapatite (HA) to the surface of titanium implants, the biomimetic[13] method is one of the most promising techniques for the production of biomaterials in body environment conditions. This method consists of immersing the substrate to be coated in a synthetic solution, simulated body fluid (SBF), with a chemical composition and pH similar to that of blood plasma and a temperature similar to that of the human body. Further, the HA obtained using this method is similar to biological HA. The advantage of this technique is to control the thickness of the deposited layer, the increase in the bond to the metal substrate, and also the surface topography that increases the host's response to the implant, thus improving earlier osseointegration. Many studies show positive results for HA and titanium[6,13,14]; however, there are few studies on laser-treated titanium implants associated with HA. Based on this, our study was conducted.

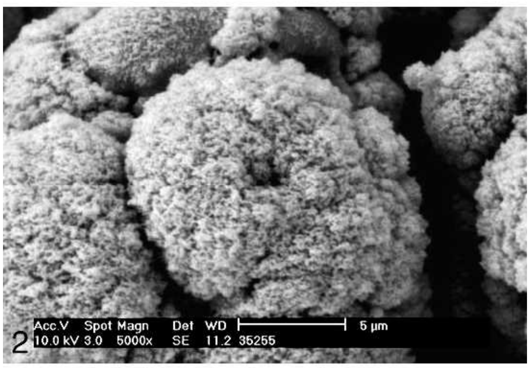
Materials and Methods

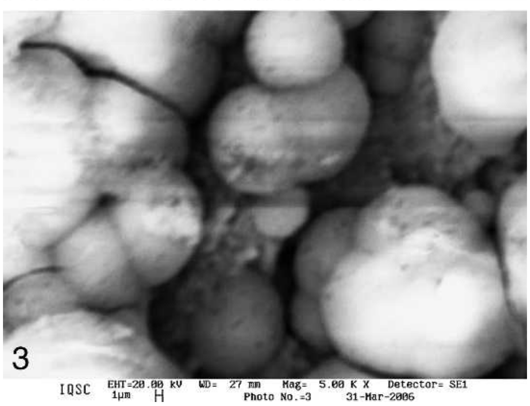
Titanium implants 3.75 $\times$ 10 mm were inserted by Branemark's method into the tibias of 30 male rabbits (New Zealand *albinus*) at 6 months age between 3 and 4 kg. The animals received 3 types of different titanium implant surface: machined (control group [GI], laser beam [GII], and laser beam + HA [GIII]). The rabbits were divided into 2 periods of observation: 4 and 8 weeks postsurgery.

Modification of implant surface

In GII and GIII, the implants were irradiated with high-intensity laser (Group of Biomaterials Institute of Chemistry, UNESP, Araraquara, SP, Brazil) with potency predetermined.[6] Following laser etching, GIII received a coating of hydroxyapatite by the biomimetic method[14] Treatment of cp-Ti with NaOH solution: GIII was washed in an ultrasonic cleaner with alcohol, acetone,







Figures 1–3. Figure 1. SEM GI: machined titanium implant surface. **Figure 2.** SEM GII: titanium implant surface modified by laser beam. **Figure 3.** SEM GIII: titanium implant surface modified by laser beam + hydroxyapatite.

and deionized water for 10 minutes. Immediately afterward, the substrates were etched in 5M NaOH solution for 24 hours at 60°C and heat treated at 600°C. Apatite coating on cp-Ti: After the treatment with NaOH solution, GIII was immersed in a solution of sodium silicate for 24 hours at 37°C. Subsequently, implants were treated with an SBF solution for slow and organized nucleation of apatite on the surface of the implants to be coated. On completion, samples were immersed for 6 days at 37°C in a solution $1.5\times$ SBF for the growth of apatite. After the deposition method, the samples were subjected to heat treatment at 600°C for 1 hour in a kiln (EDG 3P-S 1 800, EDG Equipment, São Carlos - São Paulo, Brazil).

Surface roughness analysis

The roughness average (Ra) of each type of surface was evaluated using Mitutoyo SJ-400 equipment.

X-ray diffraction analysis

The surfaces of GI-GIII were analyzed by X-ray diffraction (XRD), using an X-ray diffractometer (1 200/PC, Rigaku Americas Corporation, Salem, N.H.), to investigate crystalline composition as well as the types and phases of oxides formed. Surface characteristics were recorded using $CuK\alpha$ radiation, a 20–60° 2θ range, and continuous scanning at $0.012\theta s - 1$. Coating analyses were obtained by Rietveld refinements using the General Structure Analysis Software package computer program.

Scanning electron microscopy

All implant surfaces were analyzed using scanning electron microscopy (SEM; Institute of Chemistry, UNESP) before being inserted into the rabbits (Figures 1 through 3).

Surgery

The animals were anaesthetized with ketamine (50 mg/kg) injected intramuscularly (Vetaset, Fort Dodge Saúde Animal Ltda, Campinas, São Paulo, Brazil) and xilazine chloridrate (5 mg/kg, Dopaser, Laboratório Calier do Brasil Ltda, Osasco, São Paulo, Brazil) and received local anaesthesia, mepivacaine chloridrate (0.3 mL/kg, Scandicaine 2% with adrenaline 1:100 000, Septodont, France), to induce hemostasis. The animals also received

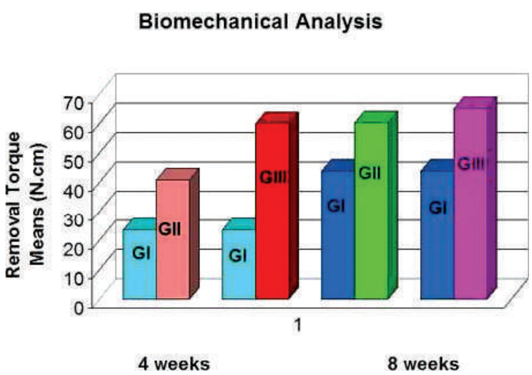


FIGURE 4. Biomechanical analysis of removal torque of GI compared with GII and GIII at 4 and 8 weeks.

antibiotic Pentabiotic (0.1 mL/kg, Fort Dodge Saúde Animal Ltda) intramuscularly postsurgery and Dipirona Sodic (1 mg/kg, Ariston Indústrias Químicas and Farmaceuticas Ltda, São Paulo, Brazil) as analgesic. The animals did not receive anti-inflammatory because it would interfere with bone response.[15] Polvidine was used as antiseptic, and the dieresis and bone perforation was carried out by the Branemark method (round drill, twist drill 2 mm, pilot 2/3, and twist drill 3 mm, with physiological solution irrigation). The implants were inserted bicortically into each proximal tibial metaphase.[16] Following the observation periods (4 and 8 weeks), the animals received overdose of anesthesia to euthanize them at the conclusion of each period.

Removal torque

After 4 weeks, half the implants were reopened, the bone and soft tissue covering the implants were removed, the mount implants were connected to the torque machine (ATG24CN Tohnich, Tokyo, Japan), and a counterclockwise movement was performed to remove the implant. The maximal torque value for breakage of bone-implant interaction was measured in Newton centimeters (N·cm), and the values were sent for statistical analysis. No forces were applied in a vertical direction so as to avoid alterations in the data.[9] After 8 weeks, the remaining implants underwent the same process.

Statistical analysis

Statistical analysis used the Student-Newman-Keuls method.

RESULTS

In GII and GIII, the surface analysis showed a morphology affected by melt and quick solidification zones following laser irradiation, resulting in spherelike structures on the entire surface. In short, the laser treatment yielded a homogenized, porous surface that increased surface area and volume. The biomechanical analysis of GI at 4 weeks showed less force at torque removal when compared with GII and GIII. At 8 weeks, there were no significant statistical differences among the 3 groups (Figure 4). Surface roughness measurements are shown in Table.

DISCUSSION

The surface of implants always causes concern and interest in scientific communities[17] because the surface has a close relationship with the time required for osseointegration. Consequently, delay between surgery and prosthesis installation over implants troubles patients and professionals alike, as implant surface microtopography deeply affects bone healing. As a result, research topics like implant surface and the bone-implant interface have gained significant importance in modern implant dentistry.

Surface modification can be realized by various methods, laser irradiation being one of them. There are studies using laser to alter the surface of implants[6–11,17]. In 2002, Gyorgy et al[11] used high-intensity

TABLE

The roughness average (Ra) of GI–GIII was calculated and is shown here

Surfaces	Ra µm
GI (Machined)	0.252
GII (Laser)	10.096
GIII (Laser+HA)	10.068

lasers to change the titanium surface of implants, and post postanalysis with SEM displayed good modification in the last layer of titanium. The study by Yue et al[18] of the alloy Ti6Al4V surface irradiated with laser to decrease corrosion and increase resistance presented a reduction of corrosion on the alloy and alteration to the surface without fractures and with relative malleability. In a comparative study using SEM and photoelectron spectroscopic excited by X-ray on rabbit femurs, Petö et al.[10] irradiated the surface of titanium implants that had been machined and implants that had received surface blasting (Al2O3) using pulse lasers (Nd:glass) and, after 3 months, compared the results with implants having a machined surface only to show that treatment with laser made an isomorphic topography and the new bone around presented a good resistance with torque, 20% greater than when compared to implants with a machined surface only.

At 4 weeks, our study achieved an increase of 73% using removal torque on implants with a surface modified by laser (Figure 4).

After 12 weeks, Hallgren et al[19] showed histomorphometrically that the quantity of calcified tissue deposited on the surface of irradiated implants was greater than the control group, having a bone-implant contact percentage of 40% for the laser-modified implants and 32% for the machined ones, and the removal torque test showed a mean value of 52 N·cm for the laser-treated implants and 35 N·cm for the machined implants. Cho and Jung[20] analyzed surfaces irradiated with laser in endosseous implants and revealed high values with removal torque 62.57 N·cm vs 23.58 N·cm on machined implants inserted into rabbit tibias after 8 weeks of surgery.

However, in our study, the removal torque values at 4 weeks were significant statistically (GI: 23.6 N·cm vs GII: 40.8 N·cm), whereas there were no statistically significant values at 8 weeks (Figure 4). The process of modifying the titanium surface of implants by laser ablation is viable, clean, and easy to administer. Furthermore, the melt and quick solidification produce uniform irregularities in the metal and can be controlled by various factors, such as parameters of the laser's sheaves and atmosphere during irradiation. As described by different studies, laser treatment seems to be a promising method for dental implants, resulting in a better and earlier onset of osseointegration. We noted that modified implant surfaces, produced using high-intensity lasers, have homogeneous irregularities that can improve osseointegration of this material. The micrograph of GII (Figure 2) displays a typical morphology with melt and quick solidification zones that are produced by laser irradiation. The treatment yields a homogenized porous surface that increases surface area and volume; additionally, it presents deeper and bigger linear defects that significantly increase contact surface when compared with GI. GII and GIII (Figures 2 and 3) display a greater roughness than GI (Figure 1), confirmed as a value of Ra, shown in Table 1. These homogeneous defects on the surface are important characteristics in endosseous implants because they optimize osseointegration. It was found that operating the laser in a normal room atmosphere and temperature produced a titanium surface with the physical and chemical proprieties desirable,[9] as was confirmed in XRD analysis of the surface of GII. Figure 5 shows the XRD diffractograms of the different surfaces tested. GI (machined) only showed peaks of metallic titanium. In GII (laser) XRD, characterization provided evidence that oxidation occurred on laser-treated surfaces and showed peaks corresponding to TiO2 and other titanium oxides resulting from the interaction of the surface with air.

Hydroxyapatite deserves merit because it is the principal mineral component of bone tissue. Hydroxyapatite is a ceramic phosphate or bioceramic, with a composition and structure similar to the mineral phase of bone and tooth.[21] Low crystallin-

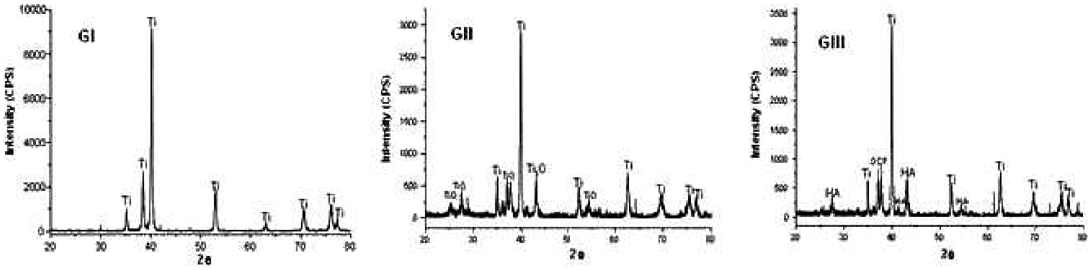


FIGURE 5. X-ray diffraction results for the machined surface GI, laser surface GII, and laser + HA surface GIII.

ity of the phases in the coating leads to instabilities when implanted. According to the literature,[22] amorphous calcium phosphates and mostly the tetracalcium phosphate and tricalcium phosphate phases are more soluble than HA, leading to rapid disintegration of the coating, known as reabsorption. GIII in Figure 5 revealed peaks of octacalcium phosphate, titanium, and carbonated HA, with more acute and well-defined peaks, characterizing greater crystallinity resulting from heat treatment.

The purpose of heat treatment used on the GIII (after deposition of HA) was to increase crystallinity and reduce solubility, thereby increasing stability of the deposition layer.[22]

The analysis by Proussaef et al[23] of implants and hydroxyapatite, after periods of function between 3.5 and 11 years, concluded that hydroxyapatite did not present absorption or dissolution regardless of the period of function and that this probably occurs only when there is contact with soft tissue. Geurs et al[24] analyzed geometrical and surface characteristics of 3 types of implant—threaded titanium plasma sprayed, threaded HA coated, and cylindric HA coated—during the process of osseointegration on a total of 634 implants and showed that HA coating tends to accelerate the initial rate of osseointegration. In a study on cellular growth using osteoblasts and biomaterials, Ramires[25] showed that there was precocious cellular aggregation, cellular differentiation, and cellular mineralization on TiO2 surfaces coated with hydroxyapatite. Balla et al[26] studied novel structures that were successfully made in Ti-TiO2 combination using laser engineered net shaping. The addition of fully dense, compositionally graded TiO2 ceramic on porous Ti significantly increased the surface wettability and hardness. Ti-TiO2 surface, modified by laser Nd-YAG to create approximately 30% volume porosity, achieved stable long-term fixation due to bone cell in-growth through interconnected porosity.

In our study, the sample GIII showed a greater surface roughness and more isomorphic characteristics when compared with GI and GII as a consequence of the presence of hydroxyapatite on the sphere-like structures. Modification of the surface of laser-irradiated implants, using hydroxyapatite, yielded an increase in surface area contact and decreased the period of osseointegration. Nevertheless, despite GIII presenting higher torque values than GII, statistical analysis indicated no significant difference (Figure 4).

On the assumption that mean roughness may be defined as the deviation of the arithmetic mean of a profile, consisting of a stable and robust descriptive parameter,[27] in the present study a direct proportional relationship was also observed between surface roughness and the values obtained by biomechanical analysis. Although in this study it is not possible to measure precisely the layer of HA, it is believed to have been less than 0.5 μm (Table).

Conclusion

Titanium implants modified by laser irradiation can increase osseointegration during the initial phase.

Abbreviations

GI: group I
GII: group II
GIII: group III
HA: hydroxyapatite
SBF: simulated body fluid
SEM: scanning electron microscope
XRD: X-ray differentiation analysis

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