HEWERSON SANTOS TAVARES[1], RAFAEL SILVEIRA FAEDA[2], ANTÔNIO CARLOS GUASTALDI[3], FERNANDO POZZI SEMEGHINI GUASTALDI[4], NILSON T.C. OLIVEIRA[5]*, ÉLCIO MARCANTÔNIO JR[6]

Unesp- Araraquara, SP, Brazil
[1]Doctoral student in Biotechnology, Biomaterials Group
[2]Doctoral student in Periodontics
[3]Associate Professor of Physical Chemistry, Biomaterials Group
[4]Surgeon Dentistry, Biomaterials Group
[5]PhD in Chemistry, Biomaterials Group
[6]Associate Professor of Periodontics

SEM-EDS and biomechanical evaluation of implants with different surface treatments: an initial study

Keywords Bone, Implant surface, Laser, SEM, Titanium.

ABSTRACT

Aim Alterations in implant surfaces can affect peri-implant bone formation and shorten the healing time. The goal of the present study was a comparative scanning electron microscopy (SEM)/energy dispersive spectrometry (EDS) and biomechanical evaluation of implants subjected to different surface treatments.

Materials and Methods Four implant surfaces were analyzed in the present study: machined commercial implants (TU); porous-surfaced commercial implants blasted with Al2O3 microspheres and acid-etched (TJA); laser beam-irradiated experimental implants (Laser) and laser beam-irradiated experimental implants with hydroxyapatite coating (HA). One sample for each surface underwent pre-surgery SEM / EDS analysis. Thirty-two implants (8 for each surface treatment) were then inserted into the tibia of 4 rabbits. After 8 weeks, the animals were euthanized and the implants retrieved by reverse torque and processed for post-surgery SEM / EDS analysis.

Results HA implants presented higher removal torque values when compared to Laser, TJA and TU groups. Post-surgery SEM micrographs clearly showed bone formation on all the examined surfaces; however, in the TU group bone covered only some areas of the implant surface, while in TJA, Laser and HA groups the entire implant surfaces were overlaid by newly formed bone. EDS analysis supported the results obtained by SEM and removal torque, showing that concentration of Ca and P increased from TU to TJA, Laser and HA implants.

Conclusions Implants with surfaces modified by laser beam with or without apatite coating showed more promising results.

INTRODUCTION

In recent years, different implant surfaces have been introduced to improve the formation of peri-implant bone and to shorten the healing time (1). The most important properties of implant surfaces are: topography, chemistry, surface charge, and wettability (2).

Microtopography has been demonstrated to be important in the bone formation process, and a higher percentage of bone-implant contact has been reported for rougher surfaces (3). In a rabbit study by Albrektsson and Wennerberg (4) it was observed that after a 4 weeks healing period, the bone volume around rough-surfaced implants was greater than in machined ones, due to a better remodeling activity at the bone-implant interface. Similar results were also reported by Zechner et al. (5) after 6, 8, and 12 weeks of contact-surface healing between implant and bone. Cho and Jung (6), evaluated the importance of different textures for machined and laser-modified implants in a rabbit tibia model. After a 8 weeks healing period, the implants were removed by reverse torque and it was found a torque value of 62.57 Ncm for laser-treated implants, while a torque of only 23.58 Ncm was obtained for the machined surface. The authors (6) concluded that removal torque values, and thus implant fixation to the bone tissue, were increased by laser beam modification of the implant surface. Suzuki et al. (7) found a higher bone volume around rough implants than in machined ones after 42 weeks of implants placement; similar results were also reported by Grizon et al. (8) after healing periods of 12 and 18 months.

Also surface chemistry has a relevant role in peri-implant bone formation. Morra et al. (9) found a clear relationship between surface composition and topography, which could be easily accounted for the chemical effects of the surface treatment performed. Indeed, an increased adsorption of Ca and P ions, proteins, lipoproteins and peptides to more hydrophilic surfaces has been described (3, 10); this fact, in turn, could influence the rate of cell attachment and spreading (10), and thus potentially enhance bone formation. It has been demonstrated that calcium phosphate materials are bioactive, forming a direct bond and a uniquely strong interface with bone tissue (11).

Alterations in implant surfaces have been proposed by several authors (12-14) in order to increase the contact surface at the bone-implant interface and to promote physico-chemical interactions leading to a greater or faster bone formation (15). The goal of the present study was a comparative scanning electron microscopy (SEM)/energy dispersive spectrometry (EDS) and biomechanical evaluation of implants subjected to different surface treatments.

MATERIALS AND METHODS

Following approval of bioethics committee for animal experimentation of the UNESP, 4 white New Zealand rabbits, 10-month old (weight 3-3.5 kg), were used in the present study.

A total of 32 wide cylindrical implants (3.75 x10.0 mm) were used: 8 machined commercial implants (TU) – control group; 8 porous-surfaced commercial implants, blasted with Al_2O_3 microspheres and acid-treated (TJA); 8 laser beam-irradiated experimental implants (laser); and 8 laser beam-irradiated experimental implants with hydroxyapatite coating (HA).

The laser irradiation procedure for Laser and HA groups was performed with the Digilaser DML 100 – Violin 10 – Nd:YVO4 equipment (ADITEC Ltda, Cravinhos, SP – Brazil) in normal environmental atmosphere using the following parameters: power 100%; scanning speed 100 mm/s; repetition rate 35 KHz; peak power 14,5 KW and fluency 280 J/cm^2 (16). After laser treatment, HA samples were coated by hydroxyapatite using the biomimetic method (17,18).

One sample for each surface underwent pre-surgery SEM/EDS analysis. The samples were cleaned in a ultra-sonic bath with deionized water for 10 minutes and pure acetone for further 10 minutes, but not metalized at this stage in order to keep the surface properties undamaged. The experiments were conducted using a SEM microscope (LEO 440, LEO Electron Microscopy Ltd, Cambridge, UK), coupled with a energy dispersive analyzer (model 760 Si(Li) with a resolution of 133 eV.

Surgical Procedure

The animals were anesthetized with a combination of ketamine (0.35 mg/kg) and xylazine (0.5 mg/kg). The initial procedure consisted in shaving with a sharp blade for skin exposure, followed by the application of an antiseptic solution. Tibial metaphyses

were then exposed by an incision in the proximal-distal direction of approximately 3 cm in order to place 2 implants into each tibia. Each rabbit received 4 implants, 2 in the left and 2 in the right tibia. The preparation of the bone site was performed with burs under abundant irrigation with saline solution. The implant insertion procedure obeyed a progressive milling sequence (19), with the motor speed reduced to 20 rpm. After the implants insertion, the cover screw was placed and the soft tissues were sutured in layers; a single dose of antibiotic (0.25 g Cefazolin, IM - Cefazolin M, Ancef, Glaxo SmithKline, Brazil) was given in the post-operative management.
After a 8 week healing period, the animals were euthanized with an overdose of anesthetic. A biomechanical test was then performed through implant removal with a manual dynamometer (15-BTG, Tonich, Japan) using a counterclockwise movement. The maximum torque value required for removal was recorded for each implant. The implants were then packaged, dried, and prepared for post-surgery SEM/EDS, following the procedure described above plus gold sputtering (Emitech K 550, Emitech Ltd, Ashford, Kent, UK).

RESULTS

Pre-surgery surface characterization

Analyzing the different surfaces by SEM and EDS before the surgical procedure, it was possible to observe differences in the surface topography and chemical composition.
TU group showed a smooth surface (Fig. 1a), while the surface irregularities increased from TJA to Laser and HA groups. By EDS only titanium was found in TU surface (Fig. 1b).
On TJA samples some surface irregularities produced by the sandblasting procedure were observed (Fig. 1c). The persistence of blasting material (residues of Al_2O_3 particles)

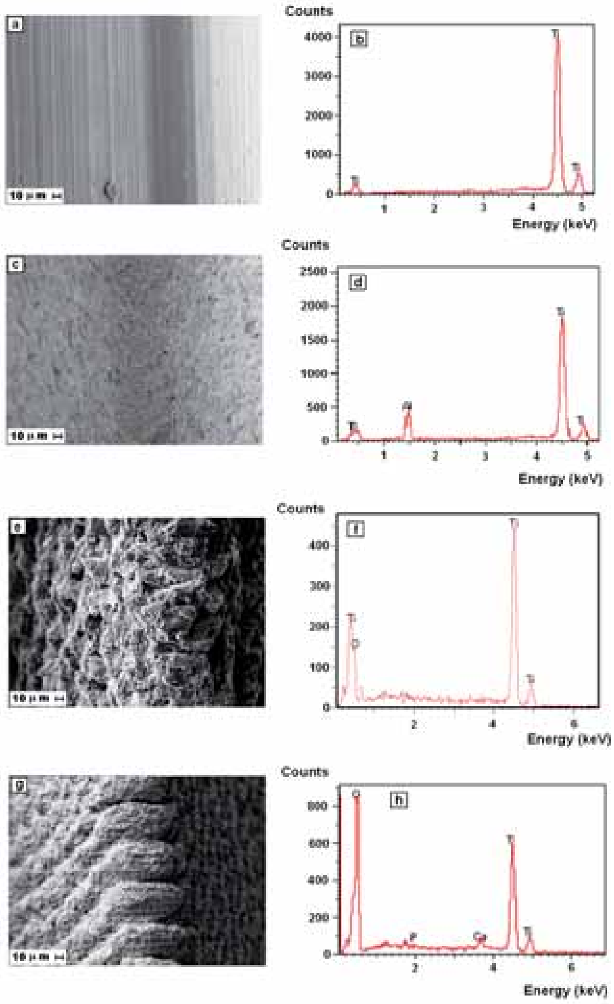

Fig. 1 Pre-surgery SEM micrographs and EDS analysis.
a Machined commercial implants (TU) showed a smooth surface.
b By EDS titanium was found in TU group.
c On porous-surfaced commercial implants blasted with Al_2O_3 microspheres and acid-treated (TJA) surface irregularities produced by the sandblasting procedure were observed.
d A peak of Al was detected on the TJA surface by EDS indicating the persistence of residues of Al_2O_3 particles.
e Laser beam-irradiated experimental implants (Laser) presented a surface with large depressions.
f A O peak was found on Laser group by EDS.
g Laser beam-irradiated experimental implants with HA coating exhibited a uniformly deposited coating.
h Peaks of Ca and P were observed, as assessed by EDS.
Magnification 1000x.

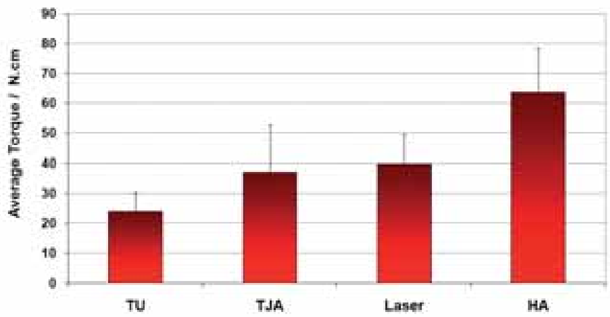

Fig. 2 Removal torque values shown by machined commercial implants (TU); porous-surfaced commercial implants, blasted with Al_2O_3 microspheres and acid-treated (TJA); Laser beam-irradiated experimental implants (Laser); Laser beam-irradiated experimental implants with hydroxyapatite coating (HA).

on the surface was supported by EDS analysis, and it was indicated by the additional peak of Al detected on the TJA surface (Fig. 1d). On the contrary, TU, Laser and HA revealed absence of contaminants.

Laser implants presented irregular shaped cavities and a typical macro/micro-structured surface with large depressions (Fig. 1e). The O_2 peak was only found on Laser (Fig. 1f) and HA (Fig. 1h) samples and was totally absent on the TU and TJA groups. This is due to the fact that Laser and HA surfaces were produced by titanium fusion in environmental atmosphere (i.e. in the presence of O_2).

HA surface exhibited a uniformly deposited coating (Fig. 1g). Peaks of Ca and P were observed even after acid etching, as assessed by EDS (Fig. 1h). Titanium concentration was lower on Laser and HA surfaces than TU and TJA.

Biomechanical Test

The average removal torque values for all surfaces was 41.17 Ncm (Fig. 2). HA implants presented the highest values, whilst TU the lowest ones.

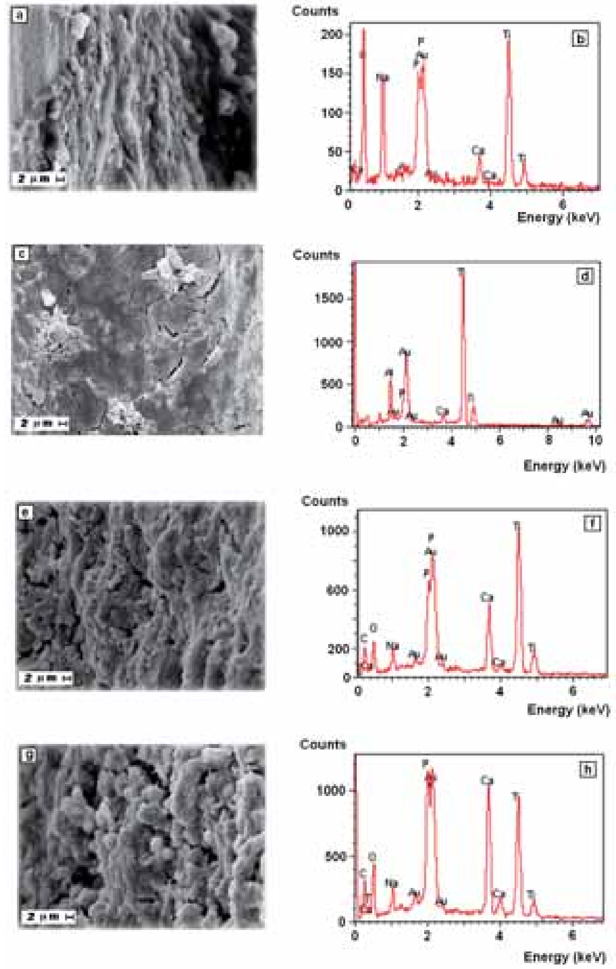

Fig. 3 Post-surgery SEM micrographs and EDS analysis.

- a In the machined commercial implant (TU), bone tissue do not uniformly cover the surface.
- b The intensity of Ca and P peaks was low in TU, as revealed by EDS.
- c On porous-surfaced commercial implants blasted with Al_2O_3 microspheres and acid-treated (TJA) newly formed bone could be observed by SEM.
- d EDS analysis showed high intensities of Ca and P on TJA group.
- e In SEM micrographs of Laser beam-irradiated experimental implants (Laser) a large amount of bone covering the surface irregularities could be observed.
- f The presence of bone could be also confirmed by the high intensity of Ca and P peaks, as assessed by EDS.
- g Laser beam-irradiated experimental implants with hydroxyapatite coating (HA) showed bone tissue in close contact to the surface.
- h HA group showed the highest intensity of Ca and P peaks by EDS.

Magnification 5000x.

Post-surgery surface characterization

Post-surgery SEM clearly showed bone formation on all the examined surfaces and it was supported by EDS indicating Ca and P peaks and thus corroborating the presence of bone tissue on all the examined surfaces (Fig. 3). In the TU group, bone tissue was only present in some areas of the surface (Fig. 3a), while in other groups it covered the entire implant surfaces. The intensity of Ca and P peaks was low in TU (Fig. 3b). Moreover intensities of Ca and P were higher in TJA than TU in all the analyzed areas of the samples. On TJA implants newly formed bone could be observed by SEM (Fig. 3c). The presence of Al could still be found in post-surgery implants from the TJA group; the Al came from the surface modification performed by Al_2O_3 blasting (Fig. 3d). In SEM micrographs of Laser group (Fig. 3e) it was possible to see a large amount of bone covering all the surface irregularities presented on pre-surgical sample surface; the presence of bone could be also confirmed by the high intensity of Ca and P peaks (Fig. 3f). HA group showed bone fragments in tight contact to the surface, suggesting that a bone to bone rupture occurred during implant removal by reverse torque (Fig. 3g), due to a strong bonding between the HA coating and the bone tissue. This group also showed the highest intensity of Ca and P peaks when compared to the remaining ones.

DISCUSSION

It has been suggested that the microtopography of the implant surface affects both the biological fixation and the mechanical anchoring of implants to bone tissue (20). Also the cleanliness of titanium dental implant surfaces is considered as an important requirement to achieve osseointegration; indeed, it has been hypothesized that the presence of contaminants could lead to failure (21). Alumina particles are widely used for blasting titanium dental implants, but blasting material may be left over on the implant surfaces, thus hampering the osseointegration process. The presence of Al peaks before and after surgery was identified by EDS on the TJA surface, indicating contamination of the implant surface (22). Although TJA implants seem to support bone formation, Ca and P concentration is lower on this surface when compared to laser treated surfaces with or without HA coating. Indeed, laser beam irradiation can be considered a clean process (16, 23). By comparing different implants surfaces obtained by mechanical processes (machining and abrasive sandblasting), chemical processes (acid etching and oxidation), thermal processes (plasma spray) and laser processes (laser beam irradiation), it has been observed that the surface resulting by laser beams irradiation shows similar characteristics without the occurrence of contaminations. Moreover, laser irradiation is a reproducible process, it enables a better control of all the parameters needed to obtain the desired surface topography (16), it shows advantages regarding standardization and ease surface treatment, and finally it is a low-cost process when compared with the other methods (16, 24).

The biomechanical analysis of implants by reverse torque was initially introduced to measure the force required to break the bone/implant interface, and therefore it was considered an indirect way of measuring implant osseointegration (12). In the present study, TU and TJA implants showed removal torque values similar to those found in the literature (4-6, 22, 25, 26), although lower than in the Laser and HA groups. Comparing the post-surgery EDS results between the laser beam-irradiated implants with or without HA coating and TJA groups, a quantitatively higher formation of Ca and P was observed for implants of the laser groups; this fact is related to

higher bone formation in those implants. Both the TJA, Laser and HA groups showed bone-like formation; however, there was no difference between TJA and Laser groups in the biomechanical test; a similar result was also found in a rabbit study by Cho and Jung (6). HA implants showed the highest removal torque with differences with the other groups; this was also supported by EDS, which showed great differences in the amounts of Ca and P in the HA group, when the pre and post-surgery HA surfaces were compared. Moreover, the HA-coated surface indicated tight attachment of bone tissue; it was observed via SEM that a bone layer remained attached to the surface of HA implants after breakage of the bone to bone tissue interface.
In conclusion, in the present study the osseointegration of 4 titanium surfaces was evaluated in a rabbit model. The results obtained suggest that, when compared with the others surfaces, laser beam-irradiated experimental implants with HA coating present a higher bone tissue formation and removal torque values, indicating a better osseointegration resulting from the physicochemical and morphological modification of the surface. Further studies will be conducted to demonstrate whether the improved bone apposition observed in this study is corroborated with superior bone anchorage at earlier time points.

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