Research article

Histomorphometric evaluation of Direct Laser Metal Forming (DLMF) implant surface in the type IV bone: a controlled study in human jaw

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Abstract

Background and objectives. Direct laser metal forming (DLMF) is a procedure in which a high power laser beam is directed on a metal powder bed and programmed to fuse particles according to a CAD file, thus generating a thin metal layer. This surface produces structures with complex geometry and consequently allows better osteconductive properties. This study evaluated the influence of two different implant surfaces on the % bone-to-implant contact (BIC%) and bone density in the human type IV bone after 2 months of unloaded healing.

Materials and Methods. The micro-implants utilized presented DLMF surface and a machined (As-M) surface serving as test and control, respectively. Sixteen subjects (67.5±4.3 years of age) received one implant each during conventional implant surgery in the posterior maxilla. After 8 weeks, the micro-implants and the surrounding tissue were removed and prepared for histomorphometric analysis.

Results. Two As-M implants were found to be clinically unstable at time of retrieval. Histometric evaluation showed significantly higher BIC% and bone density for the test compared to the control surface ($p<0.05$).

Discussion and Conclusion. The histologic data suggests that the DLMF surface implants positively modulated bone healing at early implantation times compared to the As-M, at least after 2 months unloaded healing.

Keywords. Dental implants, materials testing, maxilla, titanium.

1. Introduction

Long-term studies have shown the high predictability of dental implant supported-restorations in edentulous patients **[1,2]**. However, previous data **[3,4]** demonstrated that the survival of these dental implants placed in posterior maxilla, i.e. type IV bone, were inferior to those placed in the anterior mandible, where the bone density is higher. The demand for improved dental implant survival at sites of lower bone density has prompted

researchers to look for implant surface topography alterations that would increase the early host-to-implant response and the system temporal biomechanics.

Since the dental implant surface is the first part of the biomedical device to interact with the host, body fluids and cell interaction to micrometer scale features such grooves, ridges, and wells, as well as different chemistries have been investigated **[5,6]**. Earlier studies **[7-10]** developed by our group have demonstrated that rough implant surface topography at micrometer scale improved osteogenic response compared to machined dental implant surfaces under unloaded conditions.

Traditional methods utilized for manufacturing and processing dental implants resulted in a high-density titanium structure with a micro- or nano-rough surface. However, using these methods, it is not possible to fabricate implants with a functionally graded structure, possessing a gradient of porosity perpendicular to the long axis, a relatively high porosity at the surface and a high density in the core **[11]**.

In the last decades, considerable progress has been made in the development of rapid prototyping techniques, including direct laser metal forming (DLMF)**[7]**. DLMF is a timesaving metal forming procedure in which a high power laser beam is directed on a metal powder bed and programmed to fuse particles according to a CAD file, thus generating a thin metal layer. Apposition of subsequent layers gives shape to a desired 3D form with the need of minimal post-processing requirements **[11]**. This technology allows fabricate dental implants with different shape and texture, directly from CAD models. In addition, laser-forming methods allow the fabrication of functionally graded titanium implants, with a gradient of porosity perpendicular to the long axis. Moreover, with DLMF, a porous surface structure for bone ingrowth is provided **[7,11,12]**. However, there is few human histological information about the behaviour of DLMF implants placed at type IV bone. Therefore, the aim of this histological study was to evaluate the bone-to-implant contact (BIC%) around unloaded DLMF implants retrieved after 2 months healing from human posterior maxilla.

2. Materials and Methods

2.1. Subjects

Sixteen totally edentulous subjects (9 women; 7 men), with a mean age 67.5±4.3 years of age, referred to the Department of Periodontology and Oral Implantology (Dental Research Division, University of Guarulhos, Brazil) for implant therapy were included in this study. Exclusion criteria included pregnancy, nursing, smokers, and any systemic condition that could affect bone healing. The Ethics Committee for Human Clinical Trials at Guarulhos University approved the study protocol (CEP#201/03).

2.2. Experimental Implant Surface Topographies

In this study, screw-shaped micro-implants were prepared with 2 surface morphologies: As - machined (As-M) and direct laser metal forming (DLMF) surface. Each micro-implant was 2.5 mm in diameter and 6.0 mm long. The cpTi micro-implants were made of grade-4 titanium (Conexão Implants, São Paulo, Brazil).

The DLMF was made of master alloy powder, Ti-6Al-4V (Tixos, Leader Implants, Novaxa, Milano, Italy) with a particle size of 25-45 μm as the basic material. Processing was carried out in an argon atmosphere using a powerful Yb (Ytterbium) fiber laser system (EOS GmbH Munchen, Germany) with the capacity to build a volume up to 250 mm × 250 mm × 215 mm using a wavelength of 1054 nm with a continuous power of 200 W, at a scanning rate

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of 7 m/s. The size of the laser spot was 0.1 mm. To remove residual particles from the manufacturing process, the samples were sonicated for 5 min in distilled water at 25°C, immersed in NaOH (20 g/L) and hydrogen peroxide (20 g/L) at 80° C for 30 min, and then further sonicated for 5 min in distilled water. Acid etching was carried out by immersion of the samples in a mixture of 50% oxalic acid and 50% maleic acid at 80°C for 45 min, washing for 5 min in distilled water in a sonic bath.

2.3. Implant Surface Characteristics

The samples were first checked for chemical composition with XPS/ESCA (X-Ray Photoelectron Spectroscopy/Electron Spectroscopy for Chemical Analysis), and no significant pollution was detected **[6]**. The topographies at the microscale were then visualized using routine Scanning Electron Microscopy (SEM) control. At the nanoscale, the SEM confirmed that both surface types were nanosmooth, following the current definition **[6]**. The sole difference between these 2 tested implant types was therefore the specific surface microtopography.

An optical laser profilometer (Mahr GmbH, Brauweg 38 Gottingen, Germany) was used to measure and characterize the dental implant surface topography. Ten micro-implants from both groups (5 micro-implants from each group) were measured 3 times each on the side, top, and bottom. The measured parameters, such as the arithmetic average of all profile point absolute values (Ra), the root-mean-square of all point values (Rq), and the average absolute height values of the five highest peaks and the depths of the five deepest valleys (Rz) were measured in all specimens.

2.4. Implant Surgery

Sixteen experimental implants were used in this study (n=8 DLMF and n=8 As-M). The implants were placed under aseptic conditions as previously described **[7-9]**. After crestal incision, mucoperiosteal flaps were raised and conventional implants were placed in the totally edentulous maxilla in accordance with the surgical/prosthetic plan prepared for each patient. Next, the experimental implant groups were randomly placed in the molar region, i.e. posterior to the most distal conventional implant. The implant recipient sites were prepared with a 2.8 mm diameter twist drill in soft bone. All drilling and implant placement procedures were completed under profuse irrigation with sterile saline solution. If during placement an implant showed low primary stability, a backup surgical site was prepared. The flaps were sutured to cover the micro-implants.

Post-operative medication included Clindamicin administered three times a day (1200mg/day) for 7 days week. The sutures were removed after 10 days. To enable subjects to control postoperative dental biofilm, 0.12% chlorhexidine rinses were prescribed, twice a day for 14 days.

After a healing period of 2 months, during the 2-stage surgery of the conventional implants, the experimental implants and surrounding tissues were retrieved with a 4.0-millimeter-wide trephine bur, and the specimens were initially fixed by immersion in neutral formalin at 4%.

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2.5. Specimen Processing and Histomorphometric Analyses

Following retrieval and initial fixation, the implants and surrounding tissues were stored in 10% buffered formalin and processed to obtain thin ground sections (Precise 1 Automated System, Assing, Rome, Italy) as previously described **[13]**. The specimens were dehydrated in an ascending series of alcohol rinses and embedded in a glycolmethacrylate resin (Technovit 7200, VLC, Kulzer, Wehrheim, Germany). After polymerization, the specimens were sectioned longitudinally along the implant long axis with a high-precision diamond disc at about 150 μm and ground down to ~30 μm. Two slides were obtained per implant. The slides were stained with acid fuchsin and toluidine blue. Percentage of bone-to-implant contact (BIC%) was defined as the amount of mineralized bone in direct contact with the implant surface. The measurements were made throughout the entire extent of the implant. The bone density in the threaded area (BA%) was defined as the fraction of mineralized bone tissue within the threaded area. All threads were measured and included in the statistical analysis. The specimens were analyzed under a transmitted light microscope that was connected to a high-resolution video camera (3CCD, JVC KY-F55B, JVCs, Yokohama, Japan) and interfaced to a monitor and computer. This optical system was associated with a digitizing pad (Matrix Vision GmbH, Oppenweiler, Germany) and controlled by a software package with image capturing capabilities (Image-Pro Plus 4.5, Media Cybernetics Inc., Immagini & Computer Snc, Milano, Italy).

The mean and standard deviation of histomorphometric variables were calculated for each implant, then for each group. Mann-Whitney test was used to compare the differences of histomorphometric variables between implant surfaces. The significance test was conducted at a 5% level of significance.

3. Results

3.1. Surface Roughness Parameters

Table 1 shows the profilometry measurements. The DLMF surface showed a higher mean value for all parameters (p<0.001). The surface topography of the cpTi surface was well defined, while the DLMF surface topography had no clear orientation **(Figure 1)**.

Implant Surface Topography	Ra (μm)	Rq (μm)	Rz (μm)
As-M	0.32 ± 0.03	0.43 ± 0.02	4.20 ± 3.00
DLMF	66.8 ± 6.56	77.55 ± 11.09	358.3 ± 101.87

Table 1. Mean ± standard deviation of the As-machined (As-M) and direct laser metal forming (DLMF) profilometry. Mann-Whitney test (p<0.05).

Differences statistically significant between the implant surface topographies (p=0.0001), cpTi<DLMF; **Ra** - arithmetic average of the absolute values of all profile points; **Rq** - the root-mean-square of the values of all points; **Rz** - the average value of the absolute heights of the five highest peaks and the depths of the five deepest valleys.

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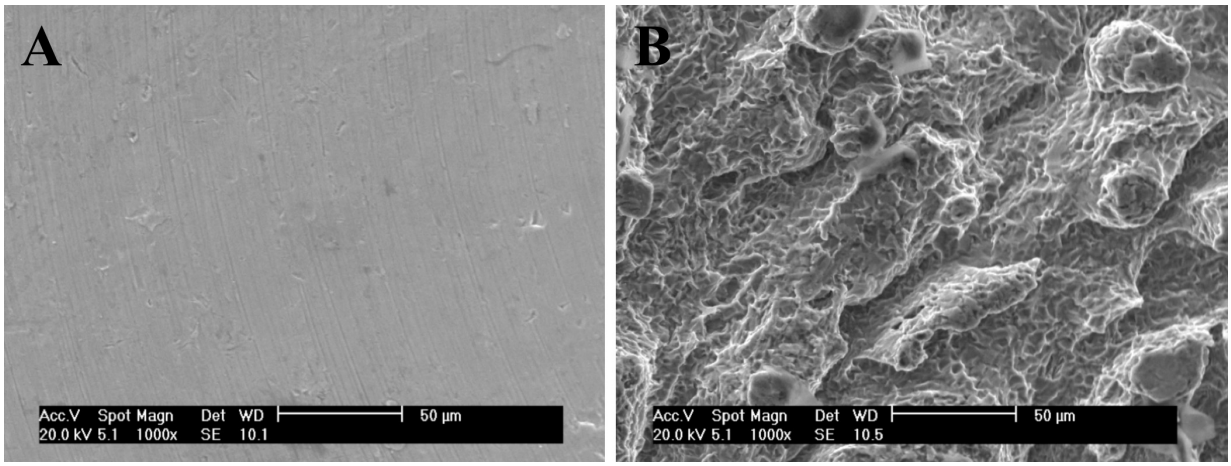

Figure 1. Scanning electron microphotograph of the evaluated implant surface topographies. (A) As-M and **(B)** DLMF.

3.2. Clinical observations

Two As-M micro-implants showed no osseointegration and were not included in the evaluation. The remaining 14 experimental micro-implants were clinically stable at the time of retrieval and did not present clinical evidence of inflammation or infection. Therefore, a total of 14 experimental implants were included in our evaluation: 8 specimens of DLMF group and 6 specimens of As-M group.

3.3. Histological and Histomorphometric Results

The pre-existing bone quality was recorded as D4 **[14]**. At coronal portion, some bone remodelling was observed in both groups. The ground sections showed the presence of remodeling activity in the bone next to DLMF implants **(Figure 2)**. Woven bone with several osteocyte lacunae and preexisting bone were present. The woven newly formed bone was separated from the preexisting bone by cement lines. The newly formed bone showed early stages of maturing and remodeling. Osteoblasts were connected to the newly formed bone, showing ongoing bone formation. Many wide marrow spaces with many capillaries were present in the peri-implant bone. In contrast, smaller amounts of new bone apposition were observed along the As-M implant surface, especially inside the implant threads **(Figure 3)**.

BIC% and BA% were statistically higher for DLMF implant surfaces **(Table 2)**.

Histometric variables	As-M	DLMF	*p*-value	CI 95%
BIC%	10.02 ± 4.53	25.14 ± 1.34	0.0001	8.65 to 25.17
BA%	17.95 ± 7.82	33.36 ± 5.90	0.003	8.58 to 39.66

Table 2. Mean and standard deviation of bone-to-implant contact percentages (BIC%) and bone density in the threaded area (BA%) for machined (As-M) and direct laser metal forming (DLFM) surfaces in posterior maxilla (n= 14 subjects). Two experimental implants from As-M were not evaluated. Mann-Whitney Test (p<0.05).

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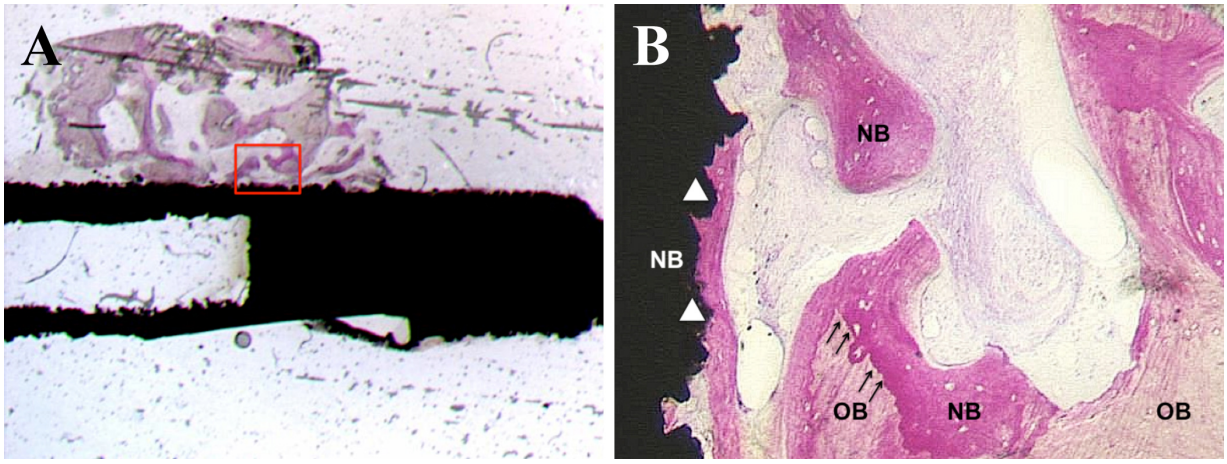

Figure 2. Histologic ground section of DLMF implant. (A) General view. The old bone was mostly lamellar (Basic fuchsin and toluidine blue staining, original magnification x20). **(B)** A larger magnification of the lateral frame area in the section shown in **(A)**. Apposition of new bone (NB) is depicted in close contact (arrow heads) with the implant surface. Reversal lines (arrows) showing the limits between old bone (OB) and new bone (NB)(Basic fuchsin and toluidine blue staining, original magnification x200).

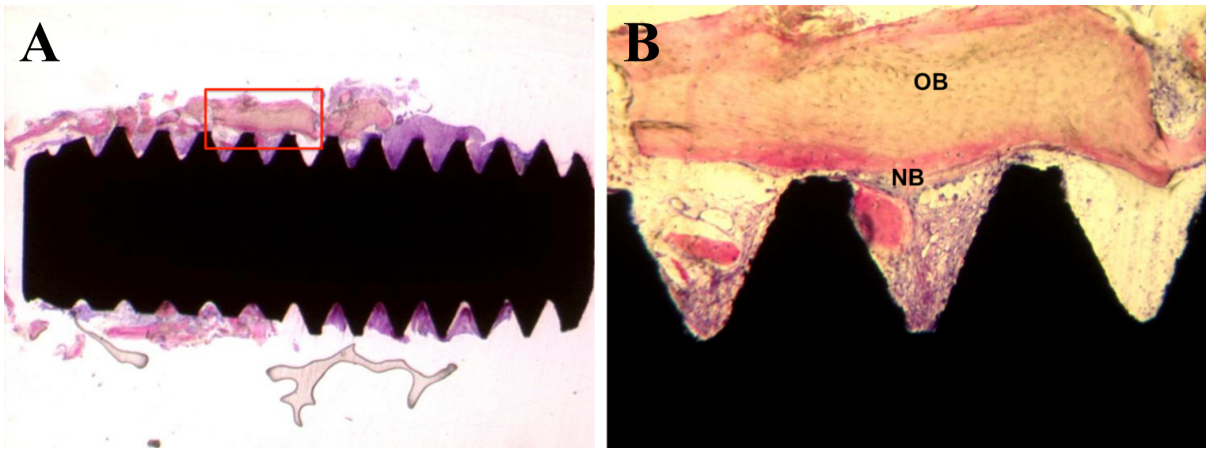

Figure 3. Histological ground section of the As-Machined surface implant. (A) General view after 2 months of healing depicting the newly formed bone showing early maturing and remodeling stages. Note the lack of connecting bridges between the new bone trabeculae and the implant surface (Basic fuchsin and toluidine blue staining, original x20 magnification). **(B)** A larger magnification of the lateral frame area in the section shown in **(A)**. The newer bone (NB) tissue shows no contact with the implant surface with presence of connective tissue (CT)(Basic fuchsin and toluidine blue staining, original x200 magnification).

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4. Discussion

This study demonstrated increased BIC% and BA% values to direct laser metal forming compared to as-machined implant surfaces. Recently, some studies have shown that DLMF influence early bone healing at the tissue/implant interface increasing bone formation in both higher and lower bone density sites **[7,15]**.

A thin bone layer covered a relatively large portion of the DLMF and micro-implant threads. This feature suggests that osteoblasts were activated by direct contact with the DLMF topography, showing contact osteogenesis **[5]**. Osteogenesis at the bone-to-implant interface is influenced by several mechanisms. A series of coordinated events, including protein adsorption, proliferation, and bone tissue deposition might be affected by the different surface topographies. At the micrometer level and beyond, the bone tissue contains complex characteristics of topographic pits, protrusions and fibers, arising in bone tissue from the nanocrystalline-mineralized osteoid. In turn, each of these events is affected by physicochemical interaction between the molecules and cells in the peri-implant area **[16]**. The implant surface chemical and topographical properties as well as the specific properties of individual proteins, determine the organization of the adsorbed protein layer.

The fabrication of dental implants with DLMF technique presents some potential advantages that could be really helpful in bone sites presenting low-density levels. DLMF makes possible to generate implants with a graded elasticity, incorporating a gradient of porosity, from the inner core to the outer surface. The outer surface of this new functionally graded material has an elastic modulus (77 Gpa) closer to that of the surrounding cortical bone (10-26 Gpa), for a more natural transfer of loading stress **[17,18]**. Complementary, extensive body fluid transport through the porous scaffold matrix is possible, which can trigger bone ingrowth, if substantial open pore interconnectivity is established **[10,19]**. Pore interconnectivity as well as pore size play a critical role in bone ingrowth regulating cell growth and function, manipulating tissue differentiation and optimizing scaffold mechanical function **[2,20]**.

5. Conclusion

Within the limits of the present controlled study, the histological data in humans confirmed that the surface topography created on DLMF implants positively influenced early bone tissue response under unloaded conditions in comparison to As-Machined surface. Further research is needed to evaluate the mechanisms of bone interaction of the DLMF surface, and to compare it with other forms of surface modifications studied in the literature.

Disclosure of interests

The authors have no conflict of interest to report.

Author Contributions

JAS was in charge of the elaboration of the study proposal and the financial support of the study, and he participated to the elaboration of the manuscript and the treatment planning of each case. CM and FM were in charge of the statistical analysis, the implant surface characterization and the financial support for the study. OB, BL and FC were in charge of the treatment planning of each case, the implant placement surgery and they participated to the elaboration of the manuscript. ST and AB were in charge of the patients´ monitoring after surgery, the biopsies collection, the oral rehabilitation and they participated to the elaboration of the manuscript. GI and AP were in charge of the laboratory

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processing of the samples, the histology, and participated to the data analyses and elaboration of the manuscript. AP also participated to the elaboration of the study proposal.

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