Early Human Bone Response to Laser Metal Sintering Surface Topography: A Histologic Report

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This histologic report evaluated the early human bone response to a direct laser metal sintering implant surface retrieved after a short period of healing. A selective laser sintering procedure using a Ti-6Al-4V alloy powder with a particle size of 25–45 μm prepared this surface topography. One experimental microimplant was inserted into the anterior mandible of a patient during conventional implant surgery of the jaw. The microimplant and surrounding tissues were removed after 2 months of unloaded healing and were prepared for histomorphometric analysis. Histologically, the peri-implant bone appeared in close contact with the implant surface, whereas marrow spaces could be detected in other areas along with prominently stained cement lines. The mean of bone-to-implant contact was 69.51%. The results of this histologic report suggest that the laser metal sintering surface could be a promising alternative to conventional implant surface topographies.

Key Words: dental implants, human histology, implant surface topography, laser manufacturing, osseointegration, wound healing

INTRODUCTION

Previous studies have shown that direct laser metal sintering (DLMS) produces structures with complex geometry that allow better osteoconductive properties. These studies also evaluated cytocompatibility and performed fibrin clot extension evaluations using osteoblasts and human whole blood to compare cell growth versus areas covered by fibrin clot. Investigators concluded that the DLMS implant...
surface presents a similar cell density to that on a rough surface but lower than that on machined surfaces. In addition, peri-implant healing is initiated immediately after implant insertion by blood clot formation. It has been hypothesized that roughened dental implant surfaces at the micrometer level might influence protein adsorption and blood clot adhesion. A previous study on DLMS depicted that extension of the human blood clot was slightly improved by inorganic acid etching to increase microroughness.\(^1\) Moreover, it was shown that implants obtained through laser metal sintering were better adapted to the elastic properties of bone.\(^2\)

Complementary DLMS implant topography minimizes stress-shielding effects and improves the long-term success rates of implants. These observations suggest that DLMS is an economical method of producing implants from commercially pure titanium or alloys. Although these in vitro data revealed some interesting results, the quantity of the bone-implant contact percentage and the quality of the bone-implant interface around these surfaces are currently under study.

Therefore, the objective of this single histologic report was to evaluate the human bone response to DLMS surface topography after a short unloaded healing period.

**CASE REPORT**

**Patient**

A 63-year-old edentulous male was admitted to our department for oral rehabilitation with dental implants. The patient was healthy and was without any significant medical history. The patient provided consent, and the study design was approved by the local Ethics Committee for Human Research.

**Implant preparation**

One screw-shaped microimplant (2.5 mm in diameter and 6.0 mm long) was prepared with direct laser metal sintering surface technology. This microimplant was made of master alloy powder Ti-6Al-4V (Leader Implants, Novaxa, Milano, Italy), with a particle size of 25–45 \(\mu\)m, 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 \(250 \, \text{mm} \times 250 \, \text{mm} \times 215 \, \text{mm}\) using a wavelength of 1054 nm with a continuous power of 200 W, at a scanning rate of 7 m/s. The size of the laser spot was 0.1 mm. To remove residual particles from the manufacturing process, the sample was sonicated for 5 minutes in distilled water at 25°C, was immersed in NaOH (20 g/L) and hydrogen peroxide (20 g/L) at 80°C for 30 minutes, and then was further sonicated for 5 minutes 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 minutes, followed by washing for 5 minutes in distilled water in a sonic bath.

The direct laser preparation provided an implant surface with a roughness surface that had the mean of the average of absolute values for all profile points (Ra), the root-mean-square of the values of all points (Rq), and the average value of the absolute heights of the 5 highest peaks and the depths of the 5 deepest valleys (Rz) of 66.8, 77.55, and 358.3 \(\mu\)m, respectively (Figure 1).

**Microimplant placement**

The patient received 1 microimplant, which was inserted between 2 implants placed in the anterior region of the mandible (Figure 2). The microimplant was placed under aseptic conditions. After crestal incision, mucoperiosteal flaps were raised and conventional implants were placed in the anterior mandible in accordance with the surgical/prosthetic plan prepared for restoring an overdenture for the patient. The microimplant recipient site was prepared with a 2-mm-diameter twist drill at 850 rpm.
All drilling and microimplant placement procedures were completed under profuse irrigation with sterile saline. The flaps were sutured to cover the microimplants. Amoxicillin was administered 3 times a day (1500 mg/d) for a week to avoid postsurgical infection. The sutures were removed after 10 days. To enable the patient to control postoperative dental biofilm, 0.12% chlorhexidine rinses were prescribed, twice a day for 14 days.

After a healing period of 2 months,3–5 during 2-stage surgery of the conventional implants, the DLMS microimplant and surrounding tissues were retrieved with a 4.0-mm-wide trephine bur (Figure 3) and were immersed immediately in 4% neutral formalin.

Histologic processing and evaluation

The ground sections were obtained by the following technique.5 The specimen was dehydrated in an ascending series of alcohol rinses and embedded in glycol methacrylate resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). After polymerization, the specimen was sectioned lengthwise along the larger axis of the implant, using a high-precision diamond disk, to about 150 μm and was ground down to about 30 μm. Two slides were obtained and stained with basic fuchsin and toluidine blue. Bone-to-implant contact (BIC%) was defined as the amount of mineralized bone in direct contact with the implant surface. The measurement was made throughout the entire extent of the microimplant. This evaluation was performed using a light microscope (Laborlux S, Leitz, Wetzlar, Germany) connected to a high-resolution video camera (3CCD, JVC KY-F55B, Milan, Italy) and interfaced to a monitor and personal computer. This optical system was associated with a digitizing pad (Matrix Vision GmbH, Milan, Italy) and a histometry software package with image-capture functionalities (Image-Pro Plus 4.5, Media Cybernetics Inc, Immagini & Computer Snc, Milan, Italy). The mean of BIC% was calculated for the implant.

Results

After a healing period, the implant presented no marginal resorption or infection. Histolog-
Histologic evaluation depicted a compact pristine bone with numerous osteocytes present in the lacunae. Areas of woven bone also could be distinguished (Figure 4). The specimen showed the presence of remodeling activity in the bone next to the microimplant. The woven newly formed bone was separated from the preexisting bone by cement lines, and several osteocyte lacunae were present (Figure 5). Histometric analyses demonstrated a bone-to-implant contact percentage of 69.51%.

**DISCUSSION**

This case report describes the histologic evaluation of cortical human bone on DLMS implant surface topography following a 2-month healing period. The DLMS surface exhibited more than 69% of mineralized bone contact after the initial 2 months of healing.

The geometric properties of the DLMS surface produced mechanical restrictions on the cytoskeletal cell components, which are involved in the spreading and locomotion of cells. The proliferation and differentiation of bone cells have been reported to be enhanced by the roughness of the implant topography surface. This phenomenon starts the recruitment of osteogenic cells from vascular connective tissue around the implant, where these cells migrate toward the implant surface topography using fibrin scaffold as migratory pathway. Osteogenic cell migration and new bone apposition allow direct bone formation on the implant surface topography. A thin bone layer covered a large quantity of the DLMS microimplant threads. This feature suggests that osteoblasts were activated by direct contact with the DLMS topography, showing contact osteogenesis.

The 3-dimensional (3D) environment of channels and pores of heterogeneous dimensions after acid etching with oxalic and maleic acids of the DLMS implant surface
topography may be conductive to bone-to-implant contact. In addition, as was previously reported, it has been suggested that acid treatment enhances early bone-implant integration to a level similar to that observed around the more complex surface topography, such as hydroxyapatite-coated surfaces and plasma-sprayed titanium. However, this single case study could not allow clear conclusions to be drawn about this phenomenon.

In addition, it may be hypothesized that faster bone formation within the cavities of the DLMS topography occurs when osteoblast precursors migrating into pores of the rough surface reach confluence earlier within the enclosed spaces, cease proliferation, and then differentiate, while growth factors may become concentrated within cavities, as is demonstrated in the case of bone morphogenetic protein and hydroxyapatite. A previous study showed that cells appeared to adhere mainly to the protruding and rounded features of the DLMS surface topography and extended between them, creating a biological architecture, which, in theory, could facilitate macromolecule concentration at the implant surface, as was previously proposed by Ripamonti. The differential cell adhesion could be related to higher surface tension in these regions than in the areas of depression, which, in turn, could influence protein adsorption and hence cell adherence.

In conclusion, this case report suggests that the DLMS implant surface results placed in dense bone tissue could be a promising alternative. However, additional controlled and prospective investigations should be conducted to evaluate several implant surface topographies.

**ABBREVIATIONS**

3D: 3-dimensional
BIC%: bone-to-implant contact

**REFERENCES**


