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Original Article

SANDBLASTED AND ACID ETCHED SURFACES: AN XPS ANALYSIS AND MICRO-CT STUDY IN RABBITS

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ABSTRACT

Current literature reports that surface acid-etching can improve osteointegration. The aim of the present pilot investigation was to evaluate the bone-implant contact of sandblasted/acid-etched surface implants through micro-CT. Two white New Zealand mature rabbits were treated in the present study, while each rabbit received 2 implants. A total of 4 implants were positioned. The animals were euthanised at 30 days from the implant positioning. The micro-CT sandblasted/acid-etched showed a mature bone appearing in close contact with sandblasted/acid-etched surface of the implant. The sandblasted/acid-etched surface appears to improve bone-implant contact at a later stage of healing.

KEYWORDS: *bone, bone-to-implant contact, machined, sandblasted, acid-etched, surface, micro-CT*

INTRODUCTION

The application of osseointegrated dental implants has been extensively introduced to support dental prostheses (1). The research on surface modifications aims to increase the bone-implant contact percentage (BIC). The past two

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decades have witnessed the development of biomaterials with surface chemical or biomechanical properties that would be expected to promote bone formation (2). Although it is generally recognised that surface characteristics represent a key factor that can influence the bone-implant contact interface, few systematic studies about this property have been investigated (3, 4). For this reason, the design of many dental implants incorporates a rough or textured, porous surface. This technical choice is justified by the concept that the implant surface roughness has been reported as an important component in enhancing the fixture osseointegration. The surface quality seems to stimulate the osteoblasts responsible for depositing the mineralised matrix. The increase of the surface area associated with this particular fixture design can produce an enhanced potential for cell attachment and tissue ingrowth around the implant surface that would be expected to provide mechanical stabilisation of the device (5).

Rich and Harris (6) created a rough surface on tissue culture polystyrene by gently sliding the round fire-polished end of a glass rod across the surface. The fibroblast cell tends to avoid surface roughnesses and accumulate at the level of the smooth parts of the tissue culture dish. In contrast, the macrophage cells seem to prefer the rough surfaces to the smooth parts. This particular macrophage behaviour has also been defined (6) as "rugophile". Similar behaviour has been found in implants in vivo; Salthouse (7) observed an affinity of macrophages for abraded Teflon implants. This tendency of roughened surfaces to attract cells of the monocytic series raises some interesting questions. Implants in contact with bone should have surfaces that attract cells of the monocytic series when it is known that osteoclasts form from cells derived from the monocyte lineage (8). The surfaces available for cell attachment can directly influence several cell characteristics, such as shape and function. In this way, it was demonstrated that the cells grown on grooved substrata are more round than cells grown on flat, smooth substrata (9–11).

A vast quantity of cellular characteristics, including the growth (12), the secretion of proteinases (13) and the gene expression (9), are influenced and induced by cell shape. Moreover, the surface texture of a dental implant can provide the potential for the specific selection of a certain cell population and profoundly influence their behaviour and function. However, detailed studies on how implant surfaces affect the cells they are in contact still need to be carried out. Therefore, the present pilot investigation aimed to assess the surfaces of the sandblasted/acid-etched implants by XPS and evaluate, in a rabbit model, the bone implant contact (BIC), bone area inner threads (BAIT) and bone area outer threads (BAOT) by micro-CT.

MATERIAL AND METHODS

Threaded sandblasted/acid-etched implants surfaces screw-shaped implants (Isomed, DUE CARRARE, Padova, Italy) have been positioned in the present animal investigation. The fixtures were placed into the tibia of 2 white New Zealand mature male rabbits according to a previously described technique (14). Each rabbit received 2 implants 4 x 10 mm. A total of 4 implants were inserted. The animals were anaesthetised by an intramuscular infiltration of fluanisone (0,7 mg/kg.) and diazepam (1,5 mg/kg.) followed by a local anaesthesia administration through 1 ml of 2% lidocaine/adrenalin solution. Therefore, a skin incision with a periosteal flap was provided to expose the tibial plate.

The preparation of the surgical sites was performed by a series of drills under copious saline solution irrigation. The periosteum and fascia were sutured with catgut and nuvafill (Ethicon Inc. Johnson & Johnson Co, Somerville, NJ, USA) sutures. No postoperative complications or deaths occurred. The animals were euthanised after a total of 30 days from implant positioning surgery. An overdose of anaesthesia was administrated, an incision was made on the tibia, and a block section was taken. All 4 implants were retrieved. The specimens were immediately stored in 10% buffered formalin, dehydrated in an ascending series of alcohol rinses, and embedded in a glycolmethacrylate resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). After polymerisation, the specimens were observed by Micro-computed tomography.

XPS Implant Surface

XPS analysed the sandblasted and acid-etched samples for detected surface composition. Briefly, the surface of titanium is covered by a thin (about 4 nm thick) oxide layer so that the maximum theoretical concentration of Ti on pure titanium is 33%, the rest being oxygen (the most stable oxide is TiO_2). Surface contamination by the adsorption of ubiquitous hydrocarbons from the atmosphere introduces a surface overlayer of carbon, readily captured by surfacesensitive techniques such as XPS, decreasing the concentration of Ti below the theoretical value (Fig.1).

Micro-CT analysis

Skyscan 1172G (Bruker, Kontich – Belgium) obtained the computed tomography scan data through a high-resolution 3D imaging system associated with an L7901-20 Microfocus X-ray Source (Hamamatsu). The radiographic volume data acquisition was obtained by a 0.5 mm Al filter applying an image pixel/size of 21.96 μm, a camera binning 4x4, a source voltage of 70 kV, a source current of 141 μA, and an exposure time of 500 ms. The final tomographic volume elaboration of the dataset scans was obtained through a built-in NRecon Skyscan dedicated software package (Ver.1.6.6.0; Skyscan Bruker). The 3D Visualisation Software package CTvox v. 2.5 and DataViewer v. 1.4.4 (Skyscan Bruker) obtained the three-dimensional reconstructions of the volume rendering and virtual sectioning views. The final analysis of the study samples was performed through the CT-Analyser (Ver. 1.13) dedicated software package.

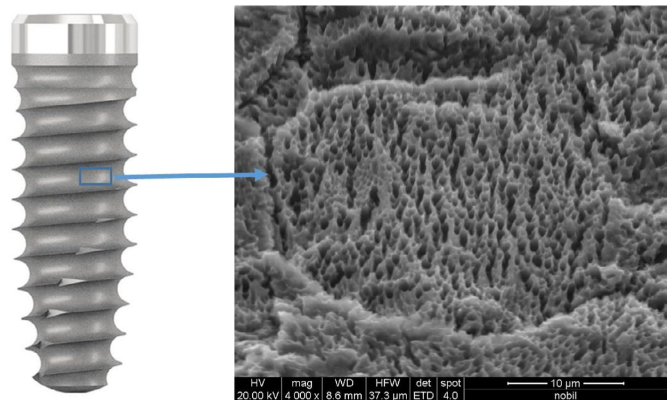

Fig. 1. *Detail of the sandblasted and acid-etched dental implant surfaces assessed in the present investigation.*

RESULTS

Micro-CT evaluation

The microtomography scans were assessed to measure the BIC, the BAIT, the BAOT and the tissue gaps at the level of the bone-to-implant interface. The micrographs reported evidence of new bone formation associated with the implant devices in intimate contact with the titanium surface. No evidence of gaps was reported after 30 days, while the BIC and BAIT measurements were more evident at the level of the implants used in this study. In addition, no aspect of local bone resorption or inflammation foci associated with evidence of osteolysis aspects were observed in the evaluated surfaces. The mean measurement of BIC percentage was 61.6±2.3%, bone area inner threads (BAIT) was 39±2.6%, and bone area outer threads (BAOT) was 43±1.3% (Fig. 2).

DISCUSSION

Endosseous dental implants positioned in the jaws with direct bone-implant contact have become a predictable option

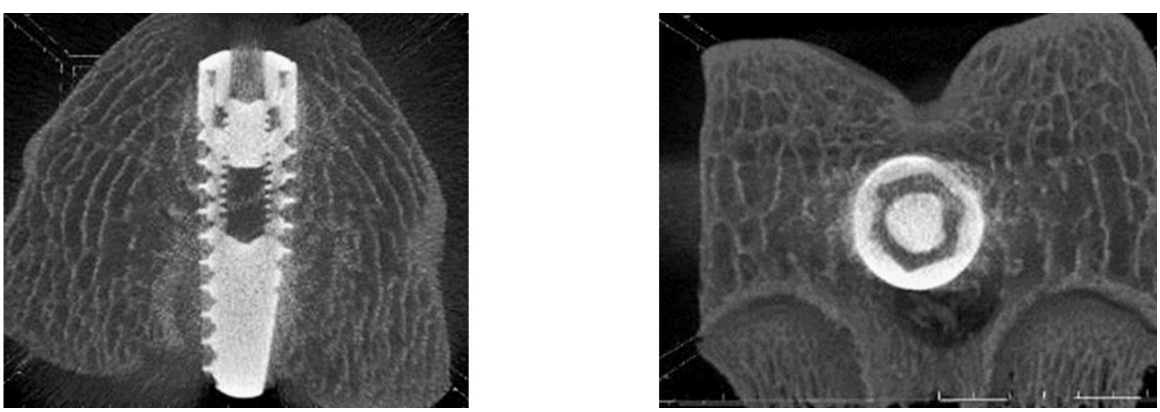

Fig. 2. *Detail of the micro-CT scans elaborated through the transection planes of the implant fixtures. The trabecular bone was in intimate contact with the implant surface.*

and widely adopted treatment for fully and partially edentulous subjects (1). The state-of-the-art includes several attempts adopted to improve the bone anchorage and mechanical stability of dental implants over time. For this scope, several variables that influenced the apposition of bone on implant surfaces have been examined. One of the most important components that could significantly affect the osseointegration of the dental implant is characterised by the surface (15). This evidence has been confirmed by Piattelli et al. (5, 16), that reported a positive correlation between the percentage of bone-implant contact and the roughness values of five different tested titanium surfaces.

The sandblasted and the acid-etched surfaces reported the best bone-to-implant contact. In this way, Martin et al. (17) have reported in an in vitro study that the marker of alkaline phosphatase activity of osteoblasts-like cells seems to be increased when associated with rough surfaces compared to smooth surfaces. The alkaline phosphatase activity is a strong indicator of bone cell maturation, while these study findings seem to suggest that the osteogenic cells in contact with an etched surface may be more differentiated than the smooth ones. These study findings suggested a significant advantage for the etched surface during the early healing period. They could also support the evidence of Kirsch and Donath (18), which reported on titanium disks with a microporous TPS surface a significantly faster bone-implant contact than smooth titanium surfaces. For this scope, the torque removal forces have been considered a biomechanical method to break the osseointegration anchorage of dental implants, and Klokkevold et al. (19) evaluated the resistance to removal forces in an animal study model on rabbits femur.

According to the present study findings, the authors concluded that the chemical acid etches treatment of the titanium fixture surface seems to potentiate the strength of the osseointegration relationship. This evidence seems to be confirmed by the reverse torque rotation values. On the other hand, Buser et al. (20) evaluated titanium implants' removal torque values in miniature pigs' maxillae. The authors compared different dental implant surfaces such as machined, sandblasted, acid etched sandblasted and acid-etched (SLA) implants. After 12 weeks of healing, the removal torque testing was performed. The authors reported that the removal torque value for SLA implants was 75% higher than the Osseotite acid-etched surface implant.

The removal torque means and histomorphometric assessment were also performed by Cordioli et al. (21) in the rabbit's tibia study model. The authors compared four different surfaces: machined, grit-blasted, sprayed, and acid-etched. After a healing time of five weeks, the histomorphometric measurements and the removal torque means were significantly higher for the acid-etched implant surface compared to the machined, blasted and plasma-sprayed surfaces. Moreover, the study findings reported that a micro-rough titanium surface submitted to an acid etching treatment procedure achieved a 33% higher bone-implant contact percentage compared to the machined surfaces. The rabbits were euthanised after 8 weeks.

The cortical bone formation was more advanced than the cancellous bone around the implant surface. A study on threaded hydroxyapatite-coated implants of commercially pure (CP) titanium was performed in an animal study on the rabbit tibial metaphysis (22). The uncoated CP titanium screw implants positioned at the contralateral leg's level were considered a control site. After 6 weeks and 1 year after the surgery, the semi-loaded implants were histomorphometrically retrieved and evaluated. While the study findings were insignificant, more direct bony contact was reported to be associated with the hydroxyapatite-coated implants after 6 weeks of follow-up. After one year from the implant's positioning, significantly more direct bone-to-implant contact was associated with the uncoated CP titanium controls. Wennerberg et al. compared the bone response of commercially pure titanium screws with two different degrees of surface roughness (23, 24).

The implants blasted with 25- and 250-µm aluminium oxide particles were positioned at the rabbit tibiae's level, and the implants' surface roughness was measured. Four weeks after the surgery, a significantly increased bone-to-implant contact for dental implants blasted with 25-µm particles was reported compared to the surfaces treated with 250-µm. This investigation indicates that a highly increased surface roughness compared to a moderately increased one is a short-term disadvantage for bone tissue. Therefore, the capability to contrast the removal forces was also compared (25, 26), considering two different surface designs of screw-shaped titanium implants.

The authors reported that the removal forces of rough implant surfaces were significantly increased compared to smooth machined implant surfaces after 6 weeks from the surgical positioning in rabbit bone tissue. Quantitative and detailed information about the orientation and shape of cells attached to the implant surface remains difficult to retrieve because the current histological techniques produce the most frequently applied thick sections (27). The tedious technique of taking serial sections and producing three-dimensional reconstruction to determine cell shape has not been employed to any great extent. Today, more studies are required to investigate how cells migrate and attach to implant surfaces and how cells would be expected to behave once they arrive there (28).

CONCLUSIONS

The results of the present investigation reported that the sandblasted/acid-etched surfaces have greater osteoconductive activity. In addition, the acid-sandblasted/etched surfaces promote increased bone-implant contact after 1 month. Concerning the early healing phase, more data have to be collected to reduce the rehabilitation time. However, further comparative animal studies are necessary to confirm the outcome of the present pilot study.

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