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Biomechanical evaluation of a titanium implant surface conditioned by a hydroxide ion solution☆

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Abstract

Two groups of titanium dental implants, identical in geometry but different in the treatment of their surfaces, were tested in an in vivo minipig model of the mandible. The surfaces that were tested were, first, sandblasted and acid-etched; and secondly, sandblasted, acid-etched, and conditioned. The removal torque was assessed at 2, 4, and 8 weeks after implantation ($n = 6$ animals in each healing period). The interfacial stiffness was also evaluated. All dental implants were well-integrated at the time of death. Removal torque values increased significantly over the course of 8 weeks. Removal torque and interfacial stiffness were increased for conditioned surfaces after 2 weeks, but there were no significant differences between the two surfaces. The sandblasted and acid-etched implants are the standard, and conditioning of the surface showed a tendency to increase early peri-implant formation of bone.

Keywords: Dental/endosteal implant; Biomechanics; Animal model

Introduction

It is widely accepted that rehabilitation of fully and partially edentulous patients can be achieved with implants of commercially pure titanium when the implants are anchored by osseointegration.[1,2] Several strategies can achieve better osseointegration. Earlier developments mainly focused on the macroscopic design of the implant, which resulted in the use of screw-shaped implants that improved both maximum primary stability and ease of handling.

The osseointegrative properties of titanium implants were improved by modifying the topography of the surface of implants. Turned surfaces were replaced by implants with increasingly rough surfaces, based on the results of in vitro and in vivo studies. Grit-blasting followed by acid etching is one of the current technologies on the market.[3] The bone–implant contact is seen as the standard descriptive measure of osseointegration.[4] Histologically, bone–implant contact increased faster and reached higher absolute measurements on rough surfaces.[5,6]

Physicochemical characteristics and biofunctional moieties of implant surfaces influence osseointegration, as do topographical properties such as macroporosity and microporosity, and are all directly linked to function.[7] Among the physicochemical surface properties, hydrophilicity and surface energy were particularly pivotal in influencing the

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host–guest response of a biomaterial and the osseointegration of medical implants.[8]

Surface energy and hydrophilicity have a pivotal role in the primary interaction of an implant with a physiological environment. This initial interaction with the surrounding hard and soft tissue is mainly mediated by the fast adsorption of a layer of protein on the surface of the implant.[9,10] The surface energy of the implant influences proteins, which are adsorbed. This primary formation of a protein film is supposed to influence directly or indirectly the whole primary, intermediate, and long-time tissue response adjacent to the host implant.[11,12] Coagulation of blood and the recruitment of inflammatory or mineralising cells or their precursors together with division, differentiation, maturation, or mineralisation might be induced with respect of the type of protein layer that forms on the surface.[13]

The aim of this study was to make a biomechanical evaluation of the osseointegration of an implant with a surface conditioned, sandblasted, and etched with thermal acid compared with that of an unconditioned control. Conditioned implants were identical to control implants, but dipped in a solution of hydroxide ions before implantation. The hypothesis was that biomechanical fixation is increased compared with that of control implants.

Materials and methods

All implants were manufactured from threaded commercially pure titanium, and were 3.5 mm in diameter and 9.5 mm long (SPI® Element, ø 3.5 mm × 9.5 mm, Thommen Medical AG, Waldenburg, Switzerland). Two different implant surfaces were evaluated: control implants (Ti) had a sandblasted and acid-etched surface (SPI® Element). Test implants (conditioned Ti) had an identical sandblasted and acid-etched surface but were also immersed in a conditioning liquid that consisted of an ultra-pure water solution containing of 0.05 M sodium ions and 0.05 M hydroxide ions. For surface conditioning the implant was dipped in a test tube that contained the conditioning solution and incubated for 20 s at room temperature.

Roughness was measured using a confocal microscope (Nanofocus μsurf, Oberhausen, Germany), over an area of 320 × 308 μm (lateral resolution 0.7 μm, vertical resolution 25 nm). The roughness values were calculated after the subtraction of the third order areal regression taking the whole area into account (not only the profiles). A Cambridge S360 electron microscope (Carl Zeiss NTS GmbH, Oberkochen, Germany) was used.

Selection of animals and the surgical protocol were approved by the commission for animal studies at the district government office, Dresden, Germany. The miniature pig model is accepted for bone healing and osseointegration of dental implants.[14,15] One hundred and forty-four implants were placed in the mandibles of 18 minipigs. Animals were randomly assigned to three groups, divided into healing periods of 2, 4, and 8 weeks. Each miniature pig was given four implants (2 conditioned and 2 controls/side) of the mandible. The positions of the implants were alternated, having been allocated using blocks of random permutations. One side was allocated to torque testing at removal, the other side to histological examination. The 8-week healing group had sequential polyfluorochrome labelling with fluorochrome applications 2, 4, 6, and 8 weeks after implantation. Histological examination and fluorochrome labelling have been reported separately.[16]

A solution of midazolam 1 mg/kg (Ratiopharm GmbH, Ulm, Germany) and ketamine 10 mg/kg (Riemser Arzneimittel AG, Greifswald, Germany), both given intravenously, was used for anaesthesia. To reduce salivation atropine 0.05 mg/kg was added to the injection. Postoperatively the minipigs were give carprofen 2–4 mg/kg subcutaneously (Rimadyl©, Pfizer Pharma GmbH, Berlin, Germany).

The mandibular primary premolar teeth were extracted. After a healing interval of nine weeks, the permanent mandibular premolar teeth were extracted under general anaesthesia and local dental infiltration anaesthesia.

After a nine weeks' healing interval, titanium implants were placed in the mandibular alveolar ridge. Minipigs were given amoxicillin 15 mg/kg intramuscularly (Duphamox©, Fort Dodge Vet.GmbH, Würselen, Germany) before operation, and anaesthetised as before. A mucoperiosteal flap was raised, and an incision made along the vestibular region, together with two releasing incisions in a perpendicular direction over the alveolar crest. The self-tapping implants were endosseously placed as described by the manufacturer's instructions. After placement implants were covered with a healing cap and tightened with a torque wrench. The flap was then repositioned using resorbable sutures.

Six animals each were killed at intervals of 2, 4, and 8 weeks and the mandibles immediately cut in half. One side was used for removal torque tests of the bone–implant interface within 12 h of death. Blocs of mandibular bone roughly 30 mm long and containing 4 implants were prepared using a diamond saw (Exakt-Apparatebau, Norderstedt, Germany). The samples were wrapped in gauze, soaked with isotonic sodium chloride, and packed in plastic bags to prevent drying out during transportation. After arrival, all soft tissue on the bone blocs was removed to expose the integrated implants and to facilitate the removal of the implant healing caps.

All removal torque tests were made on a biaxial servohydraulic material-testing machine (MTS Mini Bionix 858, Eden Prairie, MN, USA) equipped with an axial-torsional load transducer (MTS 662.20D-04, MTS Systems). The bone–dental–plaster complex was attached through the implant hexagon socket and a modified adaptor to the plunger of the actuator. The whole complex was then lowered into a slightly larger aluminium container to allow some clearance between the container walls and the block of dental plaster. The space was filled with a low-melting temperature metal alloy (Sonderweichlot, 47° eutektisch, Felder Löttechnik, Oberhausen, Germany) to ensure rigid fixation of the

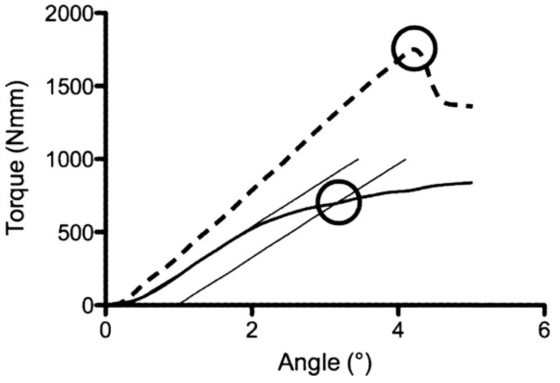

Fig. 1. Calculation of interfacial removal torque (failure). For specimen A (dashed line), the failure value (circled) is calculated from the clear peak in the torque–angle curve. For specimen B (continuous line) it is calculated from the intersection of the 0.2% offset interface stiffness line with the loading curve (circled).

Table 1
The surface topography relates to the degree of roughness of the surface and the orientation of the surface irregularities. The S_a (arithmetic mean deviation of the measured area), S_{sk} (skewness), S_{ku} (kurtosis), and α_w (surface area ratio) and S_t values of the investigated implant surfaces are given (2 samples each).

Type of implant	Ti	Conditioned Ti
S_a	2.002	2.072
S_{sk}	−0.187	−0.113
S_{ku}	2.612	2.535
A_w	1.810	1.815

specimen to the machine after the alloy had solidified, which guaranteed the alignment of the axis of the implant to the rotational axis of the actuator. The interface between the implant and actuator was not constrained vertically to eliminate any axial loading on the implant during the application of torque. The removal torque test was done by rotating the implant counterclockwise at a rate of 0.5°/s to a maximum angle of 30°. Data about angle and torque were simultaneously collected at a sampling rate of 20 Hz. After each test the alloy was melted to remove the bone–dental–plaster complex from the aluminum container. The same test was repeated for the remaining three implants of this piece of bone. The specimens were frequently sprayed with saline during testing to prevent them from drying.

The resulting torque–rotation curve was analysed to calculate the removal torque value, stiffness, and failure mode. Stiffness was defined as the best-fit linear slope of the torque–rotation curve. The removal torque (Nmm) was defined as the maximum torque recorded on the curve for specimens that showed a clear peak and subsequent drop in torque (Fig. 1, specimen A). For specimens with a constantly increasing torque–rotation curve, or a plateau in the measured torque values (Fig. 2, specimen B), a yield point was defined by constructing a straight line parallel to the initial slope of the torque–rotation curve, offset by 0.72° (0.2% full rotation), and then selecting the intersection of this offset line with the original torque–rotation curve.

The removal torque and interface stiffness (dependent variables) were analysed with the help of a factorial analysis of variance (ANOVA) with interactions for the effect of the following independent factors: type of implant surface, position of implant, and healing period. Post hoc comparisons were made using Scheffé's *F* test. All analyses were made with the help of the ANOVA/MANOVA module of Statistica 7.0 (StatSoft, Tulsa, OK, USA).

Probabilities of less than 0.05 were accepted as significant.

Results

Fig. 2a and b shows typical sandblasted and acid-etched surfaces (Ti). The macrorough surface texture caused by sandblasting can be seen clearly in Fig. 2a, and the micropits produced by the etching process in Fig. 2b. The conditioned sandblasted and acid-etched surface (conditioned Ti) shows similar topography, which is not changed by the conditioning treatment. The topographical properties of the implant surfaces are shown in Table 1.

Representative examples of removal torque compared with rotation angle behaviour are plotted in Fig. 3. The characteristics of failure at the bone–implant interface were qualitatively separated into three distinct mechanical responses: an initial "slip" of the interface followed by a substantial increase in torque values to a yield point, and torque plateau; a steadily increasing torque value until a yield point, and subsequent plateau in the torque response; or a sharply increasing torque value terminating with a single clear failure point.

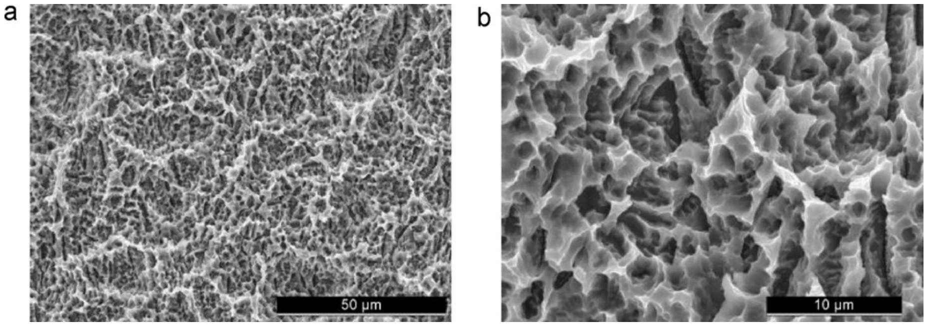

Fig. 2. Photomicrographs showing the sandblasted (a) and acid-etched (b) surfaces of a titanium implant.

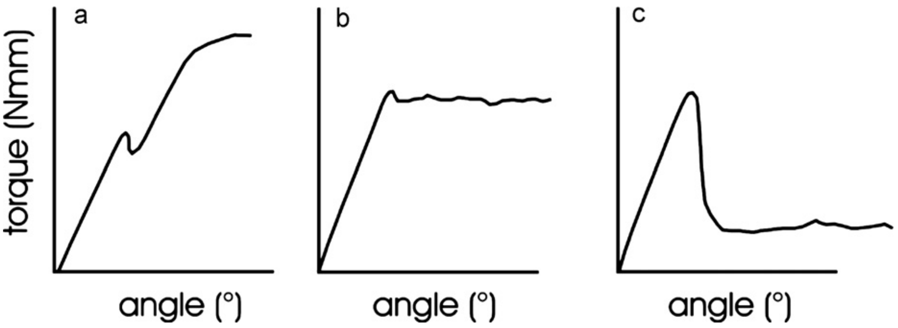

Fig. 3. Interface failure modes during biomechanical testing.

Table 2
Values for removal torque and interfacial stiffness.

Measurement/time	Ti	Conditioned Ti
Removal torque (Nmm)		
Week 2	551 (163)	604 (177)
Week 4	1336 (141)	1368 (111)
Week 8	1427 (173)	1430 (125)
Interface stiffness (Nmm/°)		
Week 2	150 (24)	166 (13)
Week 4	189 (12)	193 (21)
Week 8	186 (13)	184 (19)

All failures were characteristic of a mechanical breakdown of the osseointegrated interface. The failure of the interface in every implant corresponded to the torque curve.

Interface removal torque and interface stiffness values are summarised in Table 2, and Figs. 4 and 5. There was a substantial and significant increase in the removal torque values with increasing healing periods from 2 to 4 weeks. The position of the implant ($p = 0.63$) and its surface ($p = 0.86$) had no significant effect, in contrast to the implantation time, which had a strong influence on both removal torque and stiffness ($p < 0.001$ for each). At 2 weeks there was a possible trend towards a stiffer interface for the implants with conditioned Ti surfaces ($p = 0.09$).

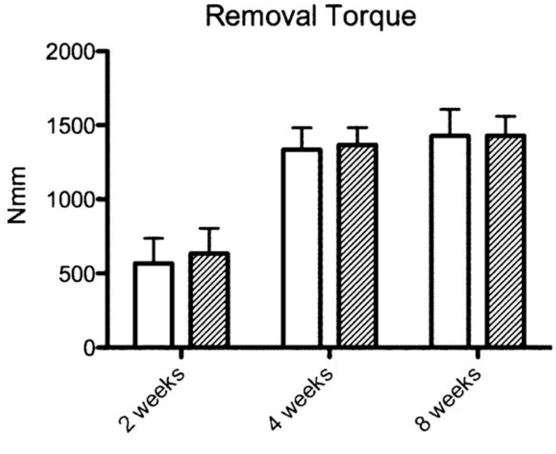

Fig. 4. Influence of type of implant surface and healing period on removal torque values. Open columns = Ti; hatched columns = conditioned Ti.

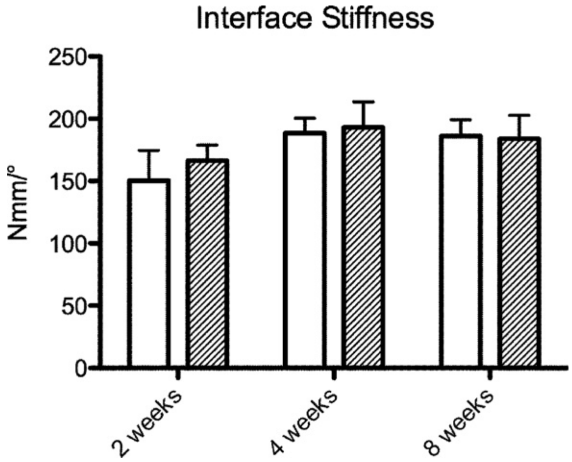

Fig. 5. Influence of type of implant surface and healing period on stiffness values at the interface. Open columns = Ti; hatched columns = conditioned Ti.

Discussion

There was a significant increase in interfacial strength with time for all surfaces tested. Interfacial stiffness increased modestly during the first 4 weeks of healing. The conditioned surface showed an improved removal torque and mean interface stiffness values at 2 weeks, but the biomechanical performance was not significantly increased compared with that of controls.

Roughened titanium surfaces are effective in improving the interfacial biomechanical properties of bone-anchored implants by providing an effective mechanical interlock.[17] Interfacial formation of bone may also be promoted by roughened surfaces, as a significantly larger degree of bone–implant contact occurs adjacent to microrough titanium surfaces than to machined or polished titanium surfaces.[5,18]

Junker et al. stated in a consensus report that there is sufficient proof that surface roughening induces a safe and predictable implant-to-bone response, but it is not clear whether this effect results from the roughness of the surface or the related change in its composition.[19]

In another consensus report, Wennerberg and Albrektsson showed that smooth ($S_a < 0.5\ \mu m$) and minimally rough ($S_a = 0.5–1\ \mu m$) surfaces responded less strongly to bone than did rougher surfaces.[20] Moderately rough ($S_a > 1–2\ \mu m$) surfaces responded more strongly than rough ($S_a > 2\ \mu m$) in some studies. In the present study, we calculated S_a values for both surfaces tested to account for a possible influence of surface topography on bony formation. The tested surfaces both showed S_a values of $2\ \mu m$, which is considered to be moderately rough.

Our results confirm high degrees of stability for sandblasted, acid-etched surfaces of titanium implants. Nevertheless, it has been suggested that implants with chemically modified surfaces that lead to more surface wetness may offer some promise.[21] For this reason, we evaluated a chemically modified implant.

The measured values imply that there is the potential for increasing the stability of the implant at the early stages of osseointegration, with mean values of removal torque 10% higher and mean interface stiffness values 11% higher at 2 weeks for the conditioned surface compared with the control.

The study had insufficient power to illustrate significance for an effect of this magnitude (statistical power of 53% for stiffness values, 16% for removal torque values). However, to achieve a statistical power of 85%, a study sample size of over 25 implants/surface and time point would be required for measurements of interface stiffness, and over 120 implants/surface and time point would be required for removal torque values. Clearly a study of this magnitude could not be supported on ethical or logistical grounds.

In a different study a significant increase of removal torque values for chemically modified implants compared with sandblasted and acid-etched ones could be shown after 2, 4, and 8 weeks.[21] The dynamic analysis showed an increase from 2 to 4 weeks on healing and a slight decrease after 8 weeks, which is explained by processes of remodelling. However, the design of the implants was different from those in our study, and implants were placed in the anterior maxilla as opposed to the mandible in our study. This limits the comparability because of the different qualities of bone between the upper and lower jaw.[22]

In a model of a sheep pelvis, removal torque values of different experimental coatings of implants were evaluated after 2, 4, and 8 weeks' healing.[7] Sandblasted, acid-etched titanium implants, calcium-phosphate-coated implants, bisphosphonate-coated implants, and collagen/chondroitin-sulphate-coated implants showed significantly higher removal torque after 8 weeks of healing, compared with surfaces modified with zirconia and anodic plasma-chemicals. The authors suggested that smaller S_a values could be a key factor for the lower torque values. There were no significant differences after 2 and 4 weeks' healing.

The implants on the opposite side of the mandible were assigned to histomorphometry. Measurements of bone–implant contact showed a tendency to increased bone formation after 2 weeks for conditioned surfaces, but not significantly so.[16] At 2 weeks, histomorphometry showed levels of 20% osteoid for both surfaces. This relatively high degree of newly formed, unmineralised bone might explain why removal torque tests were significantly lower for both surfaces at 2 weeks than at 4 weeks.

In the present animal study, we analysed the mechanical strength of the bone/implant interface of a chemically modified implant after three different periods of healing, and compared the results with those from a non-modified control. The results of the mechanical testing showed an increase in stability of the implants at 2 weeks for surface-conditioned implants, but we lacked the statistical power to verify this.

Conflict of interest statement

Bernd Stadlinger, Stephen Ferguson, Uwe Eckelt, Roland Mai, Anna Theresa Lode and Richard Loukota declare that they have no conflict of interest. Falko Schlottig is employed at Thommen Medical.

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