

Effect of micro-roughness produced by TiO₂ blasting—tensile testing of bone attachment by using coin-shaped implants

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Abstract

The aim of the present study was to examine bone response to micro-rough titanium implants. Forty coin-shaped implants were divided into eight groups according to their surface roughness. The first group had electropolished surfaces. The surfaces of implant groups 2–8 were blasted with TiO₂ particles with incremental grain sizes ranging from 7.5–12.5 to 270–330 µm. Five implants from each group were placed into the cortical bone of the proximal tibia in New Zealand Black rabbits. To avoid bone overgrowth during the retention phase the implants were fitted into tight polytetrafluoroethylene (PTFE) caps leaving only the flat test surface exposed to bone. The healing period was set to 10 weeks, and implants with attached bone were evaluated using a tensile testing machine. In groups 1–7 a significant correlation between the micro-roughness of the implant surfaces and retention strength was observed. Maximum bone bonding was observed with implants blasted with 180–220 µm grain size (group 7). Blasting with larger TiO₂ particles appeared to decrease the effect. The findings suggest that the best grain size of TiO₂ particles for optimising retention of titanium implants in cortical bone should be in the 180–220 µm range. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Coin-shaped; Titanium implants; Bone response; Surface roughness; Interfacial tensile strength

1. Introduction

A rapidly established, strong and long lasting connection between an implant and bone is essential for the clinical success of orthopaedic and dental implants. Surface roughness is one important factor in establishing such a reliable connection [1–4]. Textured implant surfaces can be produced by several methods, all of which provide different characteristics of the biomaterial surface. It is not clear what influence these characteristics have on the bone response after implantation, or to what extent the response depends on geometry and degree of the surface roughness. Results from in vitro studies indicate a positive correlation between surface roughness and both cellular attachment [5] and osteoblast-like cell activity [6]. Changes in roughness further correlate with collagen synthesis [7] and with the maturation of chondrocytes [8]. These observations are supported by in vivo studies, in particular by mechanical testing of the strength of the

connection between bone and implant [2,9]. The current knowledge concerning biological response to implant roughness achieved by histological investigations, is not clear [10,11]. It appears that a frequent tissue response to smooth implant surfaces, is formation of a fibrous encapsulation [9,10,12–17]. It has been suggested that initial stability is more likely to be achieved with implants with rough surfaces and furthermore that bone-to-implant interfacial shear strength correlates positively with the degree of surface roughness [18].

Grit blasting of titanium surfaces is a widely used method to modify surface morphology and may be used as the only treatment, or combined with additional surface modification techniques, such as acid etching. This method, according to some authors, is claimed to provide an excellent surface for bone–implant integration [10,19–21]. However, the ideal type or degree of roughness for an optimal clinical performance in bone, still remains unknown. Wennerberg [22] concluded in her thesis that an average surface roughness of about 1.5 µm, an average wavelength of about 11.1 µm and a developed area ratio of 1.5, gave the best bone fixation.

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The aim of the present study was to approach this problem and investigate bone-to-implant retention to titanium implants with different surface roughness as created by TiO₂ grit blasting. Coin-shaped implants were used, as this method presumably eliminates some uncertainties associated with most other methods [23].

2. Materials and methods

2.1. Implants

The test implants used in the present study were made from a 10 mm cylindrical bar of grade 2 commercial pure (c.p.) titanium (ASTM B 348). The implants were cylindrical and had a disc shape, with a diameter of 6.25 mm and a height of 1.95 mm.

The flat test surfaces of all discs were standardised using silicon carbide (SiC) grinding paper with successively finer grit sizes (#800 to #1200) and subsequently polished with diamond abrasive paste, 6 µm grain size, to a smooth surface according to Struers[®] Metalog Guide. The surfaces were then further modified by electropolishing or grit blasting with TiO₂ particles.

2.2. Electropolishing procedure

Eight cylindrical bars of c.p. titanium, dimension 6.25 × 40 mm, were embedded in 3,6-diazaoctan-1,8-diamin and bisphenol-A diglycidylether (Epofix resin/hardner[®], Struers, Denmark). The embedded bars were then installed in an instrument for automatic, micro-processor controlled electrolytic polishing (LectroPol-5[®], Struers, Denmark). The top of all cylinders was electropolished and then cut to give an implant thickness of 1.95 mm.

2.3. Blasting procedure

To produce surfaces with increasing roughness, one side of the disc-shaped titanium test implants was blasted with titanium dioxide (TiO₂) powders of different grain sizes. During blasting, the distance from the implants to the jets was approximately 20 mm, and the TiO₂ particle stream hit the surface at an angle of 90°. The air pressure used for blasting was set to 0.5 MPa. Blasting of each implant was performed with repeated horizontal and vertical movements during an 8 s period. A specially made titanium forceps held the test implants during the blasting procedure to prevent any contamination by other metals, and were then stored separately in glass containers.

Titanium dioxide particles with increasing grain size were used in the blasting of the different groups. The grain sizes were 7.5–12.5 µm (group 2), 22–28 µm (group 3), 45–55 µm (group 4), 70–80 µm, (group 5): 90–110 µm,

(group 6) 180–220 µm (group 7) and 270–330 µm (group 8).

2.4. Sterilisation

Prior to use all implants were treated with trichloroethylene in an ultrasonic bath for 30 min, rinsed with 100% ethanol and then treated with 100% ethanol in an ultrasonic bath for 3 × 10 min, before finally rinsing with deionised water.

The implants were thereafter autoclaved for 7 min (2 atm, 137°C) and stored separately in glass containers.

2.5. Animals

Forty-eight New Zealand Black female rabbits, 6 months old and 3.0–3.5 kg, were used in the study (Lidköping rabbit farm, Lidköping, Sweden). The animals were kept in cages during the experimental period. Room temperature and humidity were standardised.

The experiments had been approved by the Norwegian Animal Research Authority (NARA) and registered by this authority. The procedures have thus been conducted in accordance with the Animal Welfare Act of December 20th 1974, No. 73, Chapter VI, Sections 20–22 and the Regulation on Animal Experimentation of January 15th 1996.

2.6. Sedation

Ten minutes prior to removal from the cages, the rabbits were sedated by injection with fluanisone/fentanyl (Hypnorm[®], Janssen, Belgium) 0.05–0.1 ml/kg s.c.

The animals were anaesthetised with midazolam (Dormicum[®], Roche, Switzerland) 2 mg/kg bw i.v. With any signs of waking up, diluted Hypnorm[®] (1 ml Hypnorm and 9 ml sterile water) was injected slowly i.v. until adequate effect was achieved.

Lidocain/adrenalin (Xylocain/Adrenalin[®], Astra, Sweden) 1.8-ml s.p. was administered locally at the operation site.

2.7. Surgical procedures

Before surgery the operation sites were depilated and washed with soft soap and ethanol. Animals were placed on their back on the operation table, covered with sterile cloths and the operating sites were disinfected with 70% ethanol. An incision was made on the proximal-anterior part of tibiae, penetrating all soft tissue layers. The periosteum was elevated and retained by a self-retaining retractor. Four guide holes were made with a 1.0 mm diameter twist drill (Medicon[®], Germany) using a drill guide to ensure standardised and correct positioning.

Using a custom made 7.05 mm diameter stainless-steel bur mounted in a slow-speed dental implant drill with copious physiological saline solution irrigation, a platform was made for the implants. The implants were stabilised with a pre-shaped 0.2 mm titanium maxillofacial bone plate (Medicon[®] CMS, Germany), retained in the cortical bone with two 1.2×3 mm titanium screws (Medicon[®] CMS, Germany). This ensured a stable initial fixation of the implants during the healing period. Polytetrafluoroethylene (PTFE) caps were placed to inhibit bone growth towards the vertical parts of the implant as well as bone overgrowth [23]. The soft tissue was repositioned and sutured with 4-0 polyglycolic acid suture (Dexon[®] II, Sherwood-Davis & Geck, UK). Following surgery, each animal received an s.c. injection with 20 ml NaCl warmed to body temperature and buprenorphin (Temgesic[®], Reckitt & Colman, England) 0.02–0.05 mg/kg s.c. A second injection of Temgesic, also 0.05 mg/kg s.c. was given at the end of the work day—providing that at least 3 h had lapsed since the previous Temgesic injection.

Health condition was monitored during the study period and the operation sites were carefully examined daily until wound healing was complete.

2.8. Tensile test

The rabbits were euthanised using fluanison/fentanyl (Hypnorm[®], Janssen, Belgium) 1.0 ml i.v. followed by pentobarbital (Mebumal[®], Rikshospitalets Apotek, Norway), 1 ml/kg bodyweight i.v. Ten of the rabbits were chosen for the tensile test, i.e. five test implants of each group. Immediately after euthanasia, an incision was made through the soft tissue on the tibial bone. The titanium plate covering the implants was exposed and removed. A hole was made in the centre of the PTFE cap with a hollow needle, and pressurised air was applied to remove the caps and expose the reverse part of the implant. The use of this technique introduced only marginal loads to the implants. The tibial bone was fixated in a specially designed device that gave total stabilisation of the bone during the tensile test procedure. Discs machined from the c.p. titanium bar connected by a threaded pin with a ball-head were then attached to the implants with an adhesive. The set-up was then adjusted in line and perpendicular with the load-cell using a level tube. To minimise the influence of shear forces, a 300 mm long wire was connected between the load-cell and the ball-head.

The tensile test was performed with a Lloyds LRX Materials testing machine fitted with a calibrated load-cell of 100 N. Cross-head speed range was set to 1.0 mm/min. Force measuring accuracy was $\pm 1\%$ (Certificate of calibration: NAMAS Calibration No. 0019 Issued by Instron Calibration Laboratory No. 1000356). The load was applied until loosening of the implant and recorded

on a load versus time plot. Due to the design of the implant and the wider preparation of the bone the exact load needed to loosen the implant could be identified.

2.9. Surface characterisation

Scanning electron microscopy (SEM) analyses of the implant test surfaces were performed before and after implantation to record morphological details. The different sized TiO₂ particles were also analysed by SEM.

Confocal laser scanning microscope (CLSM), a Leica TCS 4D (Leica, Germany) was used to numerically describe the appearance of the surface topography. The area of measurement was $1000 \times 1000 \mu\text{m}$. The surface parameters evaluated were the average height deviation value (S_a), the maximum peak-to-valley roughness (S_t) and the developed surface area ratio (S_{dr}).

2.10. Histometric analyses

In addition to surface analysis and tensile tests, histometric measurements were performed on eight rabbits, four implants from each group. They were investigated with regard to the bone to metal contact. The bones with the implants were fixed in 4% neutral buffered formaldehyde and dehydrated before being embedded in resin (Technovit 7200 VLC[®], Kultzer & Co, Germany). Each implant was sectioned perpendicular to the long axis of the bone. The sections, approximately 1 mm thick, were polished with fine grit carborundum paper to approximately $15 \mu\text{m}$. These sections were stained with Toluidine Blue and histometric analyses were performed using a Leiz Microvid instrument. All measurements were performed at $50 \times$ magnification. Percentage bone-to-metal contact was calculated.

2.11. Statistical analyses

Statistical analyses revealing the correlation in the measured properties between groups was accomplished using Pearson's correlation coefficient.

Analyses for determination of differences in the measured properties between groups and confidence intervals were accomplished using a one-way analysis of variance (ANOVA) with a pairwise multiple comparison tests (Bonferroni).

To test the relationship between the observed tensile forces, bone-to-metal contact and different topographical parameters, linear regression was used. In this statistical set-up, tensile force measured in Newton, and bone-to-metal contact measured in percent, were used as dependent variables. The measured average height deviation values (S_a), the developed surface area

ratio (S_{dr}), and the particle size were used as explanatory variables.

3. Results

3.1. Surface analyses

Blasting of the titanium implants with TiO_2 particles resulted in a relative homogenous, rough surface structure as observed by CLSM and SEM. Increasing

grain sizes of the TiO_2 particles resulted in increased roughness of the surfaces, but caused also different topographical characteristics. The specimens in groups 2–4 had similar topographical characteristics with several small irregularities, but with increasing degree of roughness (Fig. 1). The specimen in group 8 was different, as this surface had a less homogeneous appearance at $500\times$ magnification. In several areas one could see flat facets up to $50\mu m$ in size. Between these areas there were pits, cracks and other irregularities resembling the specimens in groups 4–6. The facets

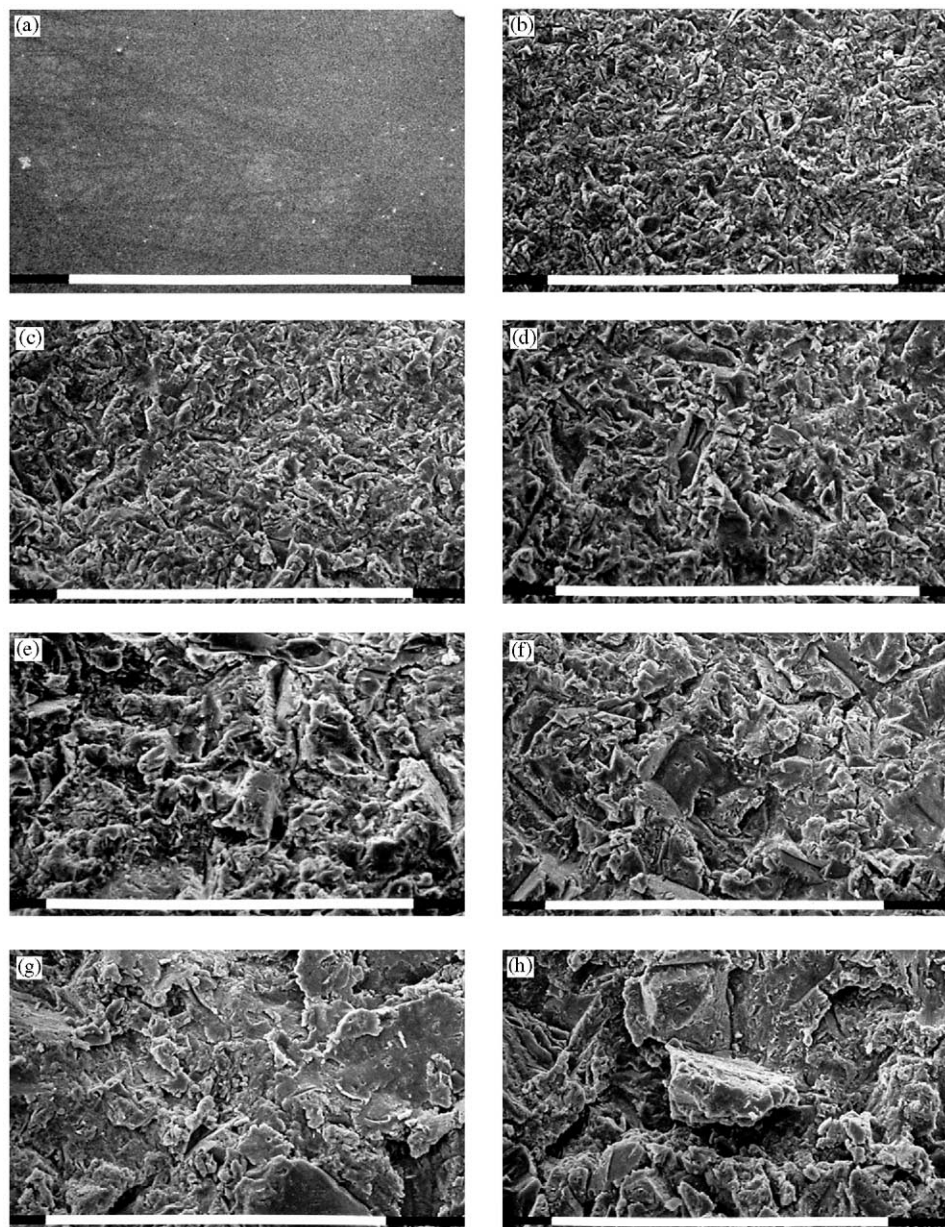


Fig. 1. Scanning electron micro-graphs of the prepared test surfaces ($\times 500$). (a) Electropolished surface; (b) surface blasted with $7.5\text{--}12.5\mu m$ particles of TiO_2 ; (c) surface blasted with $22\text{--}28\mu m$ particles of TiO_2 ; (d) surface blasted with $45\text{--}55\mu m$ particles of TiO_2 ; (e) surface blasted with $70\text{--}80\mu m$ particles of TiO_2 ; (f) surface blasted with $90\text{--}110\mu m$ particles of TiO_2 ; (g) surface blasted with $180\text{--}220\mu m$ particles of TiO_2 ; and (h) surface blasted with $270\text{--}330\mu m$ particles of TiO_2 (Bar = $100\mu m$).

also had small irregularities in the form of pits and stripes, identified at a magnification of $500\times$. The electropolished surfaces had a completely different surface topography compared with the grit-blasted surfaces. These surfaces were smooth with no irregularities that could be identified by SEM.

The geometry of the TiO_2 particles seemed to be similar within the different groups as observed at $1000\times$ magnification. All particles were edged, irregular, and with flat areas (Fig. 2). The CLSM analyses revealed a significant correlation between grain size and S_a , and between grain size and S_{dr} at the 0.01 level

(2-tailed). Pearson correlation was 0.973 and 0.907, respectively (Table 1).

The surface analysis, EDX, showed that all surfaces consisted of only the elements titanium, oxygen and carbon. Thus, blasting treatment did not result in any retention of particles, as observed by SEM. These particles were probably eliminated by the subsequent rinsing and sterilisation procedure.

Following the tensile tests, spots of calcium and phosphorous were identified on the surfaces by EDX. The presence of Ca and P was more frequently observed on the rough surfaces.

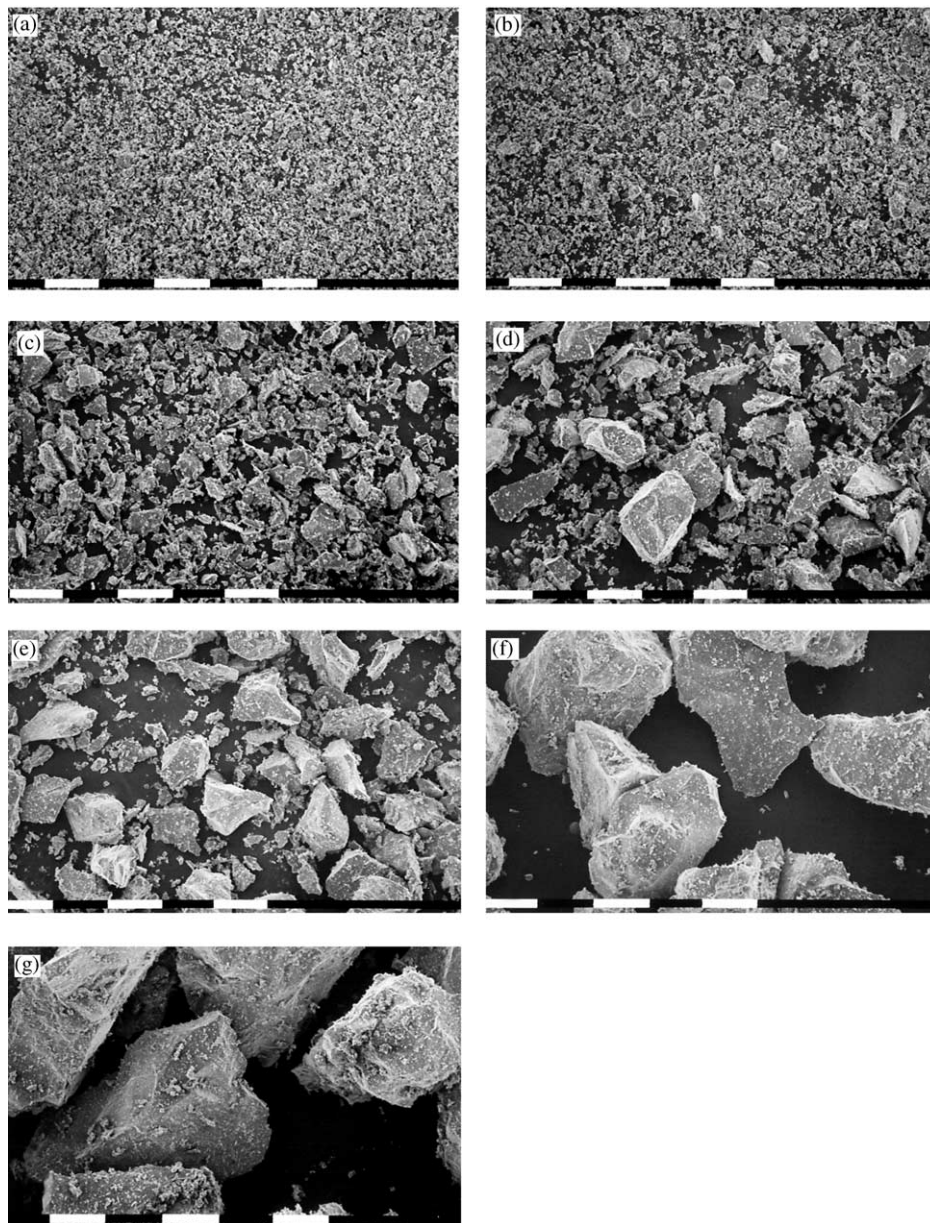


Fig. 2. Scanning electron micro-graphs of the TiO_2 grains used for blasting the surfaces ($\times 1000$). (a) TiO_2 particles with grain size $7.5\text{--}12.5\text{ }\mu\text{m}$; (b) TiO_2 particles with grain size $22\text{--}28\text{ }\mu\text{m}$; (c) TiO_2 particles with grain size $45\text{--}55\text{ }\mu\text{m}$; (d) TiO_2 particles with grain size $70\text{--}80\text{ }\mu\text{m}$; (e) TiO_2 particles with grain size $90\text{--}110\text{ }\mu\text{m}$; (f) TiO_2 particles with grain size $180\text{--}220\text{ }\mu\text{m}$; and (g) TiO_2 particles with grain size $270\text{--}330\text{ }\mu\text{m}$ (Bar = $100\text{ }\mu\text{m}$).

Table 1

Confocal laser scanning measurements of the eight surfaces. The values are mean (SD) of five scanning on two different coins in each group

Group	Surface treatment	S_a (μm) ^a	S_t (μm) ^b	S_{dr} ^c
1	Electropolished	0.63 (0.08)	15.44 (17.08)	1.02 (0.01)
2	7.5–12.5 μm TiO ₂	0.89 (0.06)	32.91 (20.70)	1.40 (0.04)
3	22–28 μm TiO ₂	1.25 (0.02)	39.29 (4.27)	1.52 (0.03)
4	45–55 μm TiO ₂	1.30 (0.06)	42.29 (6.61)	1.54 (0.05)
5	70–80 μm TiO ₂	1.80 (0.14)	59.90 (10.21)	1.64 (0.04)
6	90–110 μm TiO ₂	2.02 (0.21)	50.04 (3.11)	1.75 (0.08)
7	180–220 μm TiO ₂	3.62 (0.56)	76.28 (6.04)	2.08 (0.15)
8	270–330 μm TiO ₂	5.52 (0.74)	84.28 (6.37)	2.68 (0.57)

^a Arithmetic mean deviation of the surface.

^b Maximum peak to valley height of the surface.

^c Developed surface area ratio.

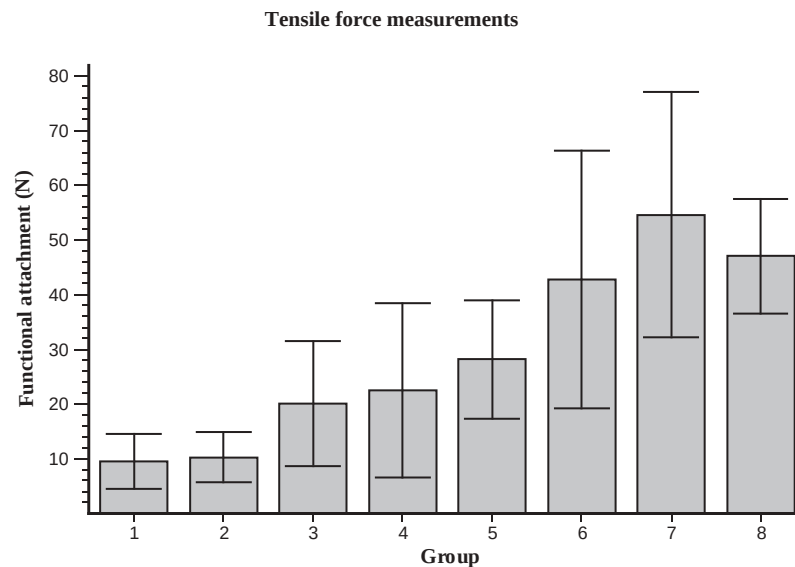


Fig. 3. Results of the tensile test measurements of the eight tested surfaces following a 10-week healing period. Bars represent mean values $\pm$ SD of five measurements of the same surface.

3.2. Retention analyses

Generally, increased implant retention correlated positively with increased surface roughness. The significance of the observed differences was determined using ANOVA and Bonferroni Post Hoc tests. Groups 4 and 5 did not differ significantly from the other groups. The group with highest mean retention (group 7) was significantly different from group 1 ($p = 0.0013$), group 2 ($p = 0.0034$) and group 3 ($p = 0.0274$).

Analysis of the results from the pullout test showed that there was a significant correlation between surface roughness (S_a) and the functional attachment ($p < 0.01$) (Fig. 3). Pearson correlation coefficient was 0.616. The area ratio (S_{dr}) also correlated significantly with the functional attachment, with a Pearson coefficient of 0.635.

In the present study the positive correlation between increasing surface roughness and functional attachment levelled out at surfaces blasted with 200 μm grains (group 7). A further increase in roughness did not give an additional improvement in implant retention.

3.3. Histometric analyses

There was no significant correlation between the degree of surface roughness, and the bone-to-metal contact, in the groups blasted with TiO₂ (Fig. 4). A reduced bone-to-implant contact was, however, observed in the electropolished implants (group 1), but this was only significantly different from group 4. The mean bone-to-implant contact varied from 26% in group 1 to 74% in group 4.

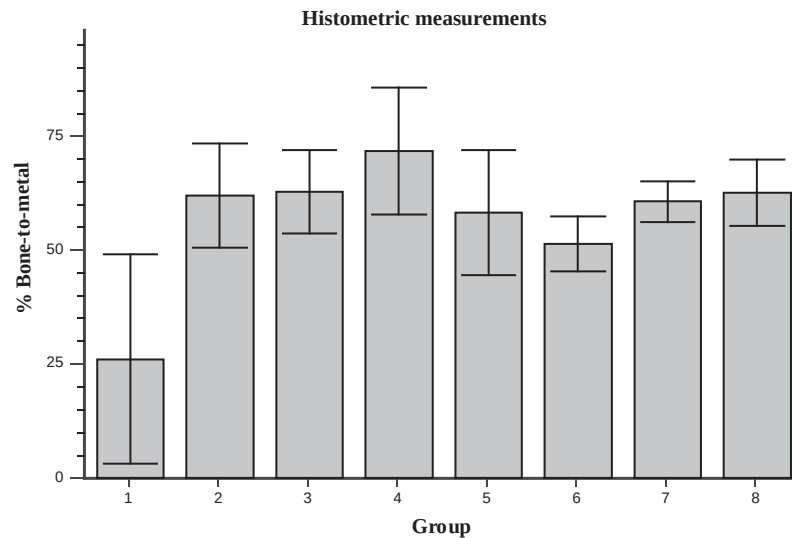


Fig. 4. Bone-to-metal contact for the eight different groups measured as per cent. Each bar shows mean values $\pm$ SD of five measurements of the same group ($n = 4$).

4. Discussion

A rough implant surface has previously been shown to be of importance for bone-to-implant retention. This has been demonstrated in animal experiments by pull-out, push-out and torque studies [3,24–31]. The association between the different characteristics of the surfaces as described by S_a values, wavelength and developed area is more uncertain. An optimal surface roughness that can be described in conventional terms has been suggested [22]. The focus of the present study was to compare the biological response of titanium implants blasted with TiO_2 particles of increasing grain size.

The shape and the fracture toughness of the particles are considered to be of significance for the outcome of the blasting procedure. The present study showed a positive correlation between increasing grain size of the TiO_2 particles, and the degree of functional attachment. This correlation was true for grain sizes between 7.5 and 220 μm . Blasting with coarser particles did not further improve the retention capacity of the implants. On the contrary, these implants appeared to have a reduced retention. This could indicate that the optimal grain size of TiO_2 particles is about 200 μm . The coarser particles used in group 8 produced surfaces with higher S_a values, but these surfaces also exhibited different surface characteristics. SEM analysis of the titanium implant surfaces showed a heterogeneous appearance, with rough parts and some less rough areas. In addition the rough areas had some smoother parts with less micropores. These observations may be of importance for an increased understanding of the mechanisms of the biological response following an implantation.

The present model was chosen because it minimises the influence of mechanical interlocking [23]. Due to the

flat, coin-shaped design of the implants, the influence of shear forces as well as the interlocking effect, are limited. By preventing bone from growing onto the vertical faces by using the PTFE caps, the frictional forces of these parts were eliminated. A certain interlocking effect between bone and metal may still occur also in this model, but will presumably have less influence on the test results than in conventional test systems.

The major aim concerning implant research is to improve the fixation of the implant in the surrounding bone-by-bone bonding or bone ingrowth. Mechanical testing of the fixation that measures the actual strength of the bonding between the implant and the bone, is thus preferable [32]. The tensile test system used in the present study is sensitive and minimises friction forces during the mechanical testing [23]. The observed retention levels can be considered to be the retention between two flat surfaces, and the observed differences in the degree of retention probably reflects the direct significance of the individual surface morphologies. Corresponding results have been reported in previous studies where blasted surfaces were found to be better than machined surfaces [11].

Histological data has also been presented indicating that rough surfaces induce an improved bone response [33]. Buser et al. demonstrated in histometric analyses significant differences in the percentage of bone-implant contact, and concluded that the extent of bone-implant interface is positively correlated with an increasing roughness of the implant surface [2]. It has also been claimed that histological measurements of bone-to-metal contact do not necessarily correlate with the mechanical evaluation of implants [25]. The histometric findings of Wong et al. stated that the correlation between average roughness and percent surface coverage

of bone was weak [34]. No correlation was observed between the histometric examination and the mechanical test in the present study. However, other authors have found such a correlation [2,11,35].

The present and previous studies indicate that histometric measurements of implant–bone interactions only give descriptive information and should be used as a supplement to mechanical measurements. Mechanical retention measurements provide direct information concerning the functional attachment between an implant and bone, with probable direct clinical consequences. Histometric analyses may however give important information about the nature and development of functional attachment of micro-rough implant surfaces in bone. The results of the present study indicate that the use of a coin-shaped model and a tensile test could be a more sensitive method than histometric measurements alone for revealing small variations in bone response to an implant surface.

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