Bone Formation at Titanium Implants Prepared with Iso- and Anisotropic Surfaces of Similar Roughness: An in Vivo Study

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ABSTRACT

Background: Implant surface topography influences the bone response after implantation. However, the importance of surface orientation is not known.

Purpose: The aim of this study was to investigate the bone tissue response and the stability of titanium implants prepared with isotropic and anisotropic surfaces of similar roughness.

Materials and Methods: A total of 18 implants were divided into two groups and were inserted into the femurs of nine rabbits for 12 weeks. Confocal laser scanning microscopy was used for the topographic description to verify that the two different surfaces were modified as intended. The stability of the implants was recorded by resonance frequency (RF) measurements at insertion and at time of removal, after which the implants were evaluated histomorphometrically.

Results: RF measurements showed that implant stability increased with time. However, there was no significant difference between the two different surface modifications at insertion and after 12 weeks. The histomorphometric comparison revealed no statistically significant differences in regard to either bone-to-metal contact or bone area inside the threads.

Conclusion: Titanium implants prepared with isotropic and anisotropic surfaces of similar roughness integrate similarly to bone during the 3 months after implantation.

KEY WORDS: anisotropy, isotropy, surface modification, surface roughness, titanium implants

Material compatibility, surface macro- and microstructure, status of implant bed, surgical installation trauma, and prosthetic loading have been established as factors controlling osseointegration.[1]

In the literature much attention has been given to changes of implant surface topography to stimulate the tissue response and to an improved and high degree of bone-to-implant contact.[2–5] Surface topography is determined by surface orientation and roughness, and it consists of form, waviness, and roughness (ie, what is left when errors of form and waviness are removed).[6] Numerous parameters exist for describing surface roughness numerically; however, not all of them are needed for routine evaluation.[7] Wennerberg and Albrektsson[8] suggested that at least one of each height, spatial, and hybrid parameter should be presented in a topographic description.

Parameters used in endosseus implant research thus far have mostly been descriptive of height, and many studies have concluded that a relation exists between surface roughness, described in values representing surface departures from a mean plane within the sampling area (S_a) or in R_a values, and the degree of implant bone integration or anchorage.[9–12] However, some results have indicated that surface orientation (isotropy or anisotropy), not roughness per se, may be responsible for the outcome with enhanced bone response.[13]

So the question remains as to whether it is surface orientation (isotropy or anisotropy) or roughness

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that causes the enhanced bone response. The aim of this study was therefore to investigate the relative importance of these two surface properties. We addressed the issue by comparing implants manufactured or prepared with a clear surface orientation (anisotropy) with implants manufactured or prepared with no dominating direction (isotropy) but with a similar roughness.

MATERIALS AND METHODS

Implant and Surface Modification

A total of 18 commercially pure turned titanium implants (Nobel Biocare AB, Göteborg, Sweden) were used in the present study. The nominal outer diameter of the implants was 3.75 mm, and the pitch height was 0.6 mm.

Nine of the turned standard implants were kept as controls (Figure 1). The remaining nine implants were blasted with titanium oxide particles of a medium grain size (7.5–12.5 μm), with the aim of creating an isotropic surface (Figure 2) that differed from that of the anisotropic turned controls. The air pressure during the blasting process was 0.35 MPa.

A TopScan 3D® profilometer (Heidelberg Instruments, Heidelberg, Germany) was used to topographically describe the two different surfaces used in this study. This measuring equipment is based on the confocal laser scanning principle. Three screws of each surface modification were topographically characterized. Each screw was measured on nine sites on the threaded area (three tops, three valleys, and three flanks). Each measurement was performed over an area of 245 × 245 μm. A gaussian filter (50 × 50 μm) was used to separate roughness from errors of form and waviness, as recommended in 2000 by Wennerberg and Albrektsson.[8]

The following parameters were measured:

- S_a: the arithmetic mean of the absolute values of the surface departures from a mean plane within the sampling area, expressed in micrometers

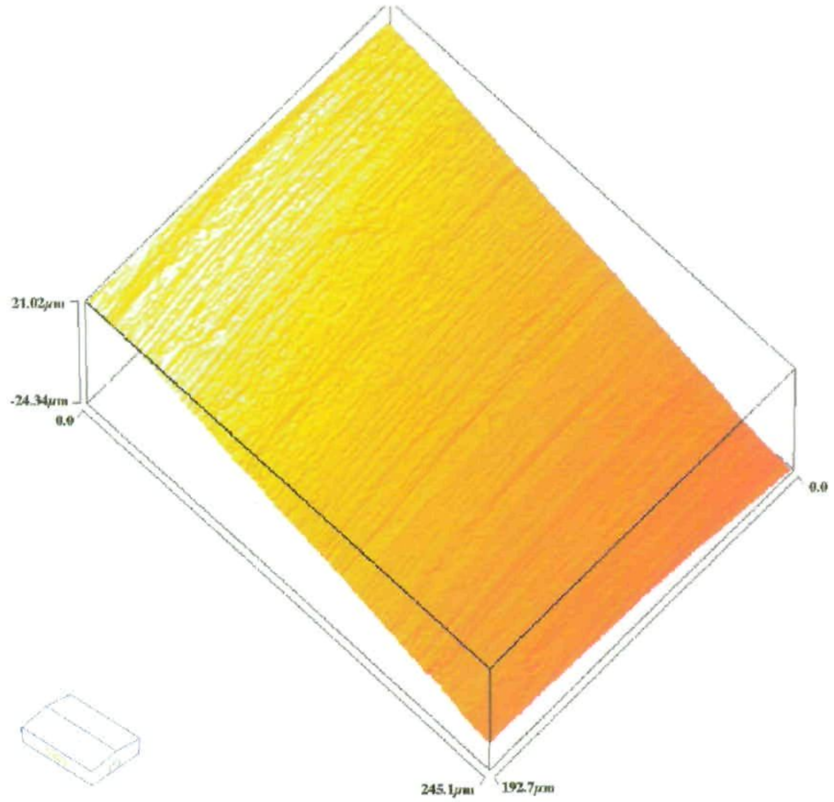

Figure 1 A turned flank. Marks produced by the cutting tool during turning are clearly visible. These marks give the surface the dominating direction of the irregularities perpendicular to the long axis of the implant.

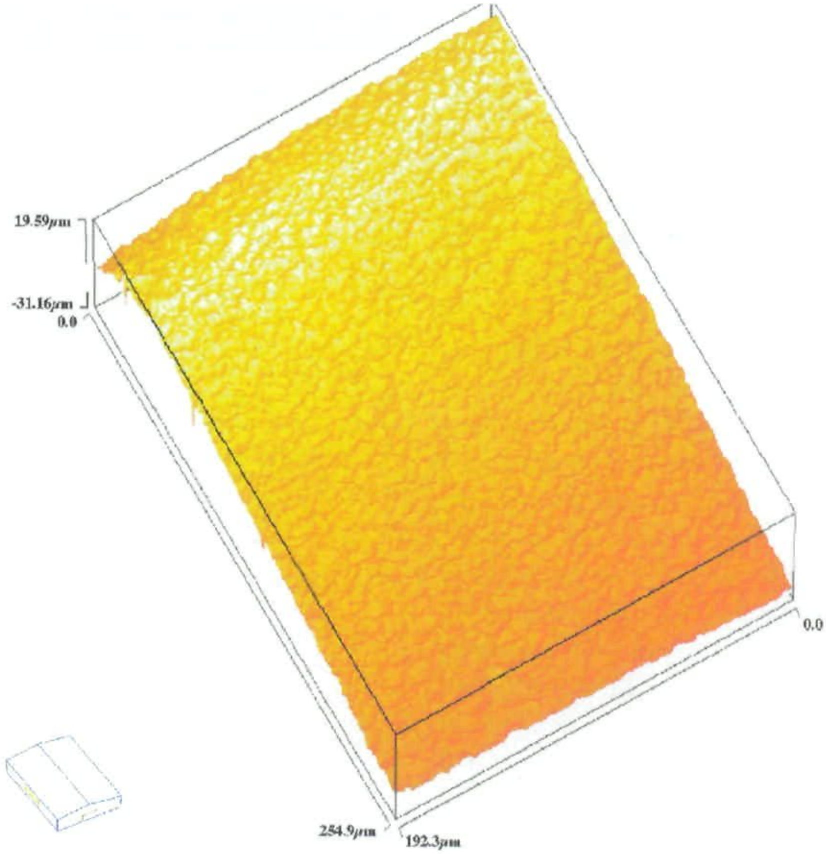

Figure 2 A turned and blasted flank. The blasting procedure has removed the turning marks, and the surface no longer has a dominating direction.

- S_{cx}: the average mean spacing of profile height in the x-axis (horizontal) direction, expressed in micrometers
- S_{dr}: the developed surface area expressed as a percentage and calculated from the measured surface and totally flat reference area

The blasting procedure was performed in such a way that the S_a and S_{dr} values of the turned implants and the blasted implants would be similar. However, the S_{cx} values were expected to differ between the two groups because of the regular traces of the turning procedure (these were supposed to be removed after the blasting process). One height, one spatial, and one hybrid parameter were used to numerically describe the roughness.

Animals and Surgical Technique

Nine adult female New Zealand White rabbits were used in the experiment. They were kept in one specially designed room. The rabbits had free access to tap water and were fed with standard pellets. Immediately after implant insertion the animals were put into separate cages to control the healing process; as soon as wound-healing circumstances allowed, the rabbits were free to return to the specially designed room. At surgery general anesthesia was induced by intramuscular injections of 0.9 mL of combined fentanyl citrate 0.315 mg/mL and fluanisone 10 mg/mL (Hypnorm®, Janssen Pharmaceutical Ltd, Oxford, UK) and intraperitoneal injections of 0.5 mL of diazepam 5 mg/mL (Stesolid Novum®, A/S Dumex, Denmark). If necessary during surgery additional doses of Hypnorm and Stesolid Novum were administered. The hind legs were shaved, and antibiotics were administered prophylactically. Immediately before surgery 1 mL of lidocaine (Xylocaine®, AstraZeneca, Södertälje, Sweden) was injected at each insertion site. The operation was performed under aseptic conditions. The holes were drilled at a low rotatory speed and under copious saline irrigation. One test and one control implant were inserted into the distal condyles of the left and right femurs, respectively.

In Vivo Evaluation

All implants were removed en bloc with the surrounding bone tissue, fixed in 4% neutral buffered formaldehyde, dehydrated in alcohol solutions, and embedded in a light-curing resin (Technovit® 7200 VLC, Heraeus Kultzer GmbH & Co., Wehrheim, Germany). Cutting and grinding was performed as described by Donath in 1988.[14] The final sections were approximately 10 μm thick and were stained with toluidine blue. Morphometric measurements were made with a light microscope and a Leitz Microvid® unit (Ernst Leitz GmbH, Wetzlar, Germany). The percentage of bone-to-metal contact and the percentage of bone inside the threaded area were calculated. Results were presented as the mean value for all threads around the implant and the mean value for the three best consecutive threads. All light microscopic calculations were made with an objective of × 10 magnification and an eyepiece of × 10 magnification.

Resonance Frequency Analysis

To measure changes in implant stability during healing, resonance frequency analysis was used. This method was described in 1997 by Meredith and colleagues[15] and gives quantitative data on the stiffness of the bone-implant interface. A small transducer was attached to the implant immediately after insertion and immediately before the sacrifice of the rabbits. Measurements were performed parallel and perpendicular to the long axis of the femur. A mean value of one parallel and one perpendicular measurement was calculated for each implant.

TABLE 1 Mean Surface Roughness

	S_a (μm)	S_{cx} (μm)	S_{dr} (%)
Turned surface	0.70 ± 0.1	9.40 ± 1.7	21 ± 7
Blasted surface	0.78 ± 0.2	10.25 ± 1.5	20 ± 6
p Value	ns	.009	ns

ns = not stated; S_a = arithmetic mean of the absolute values of surface departures from a mean plane within the sampling area; S_{cx} = average mean spacing of profile height in the x-axis (horizontal) direction; S_{dr} = percentage of developed surface area, calculated from the measured surface and the totally flat reference area.

TABLE 2 Mean Surface Morphometry*

	BMC (%)	BMC3 (%)	BA (%)	BA3 (%)
Turned surface	35 ± 11	48 ± 15	73 ± 9	85 ± 7
Blasted surface	42 ± 10	52 ± 10	70 ± 13	82 ± 5

BA = bone area inside the implant threads; BA3 = average value of the best three consecutive-thread areas inside the threads; BMC = bone-to-metal contact; BMC3 = average value of the bone-to-metal contact of the best three consecutive threads.

*For the different surface modifications, the percentages and standard deviations of bone-to-metal contact (BMC) and bone area inside the threads (BA) were calculated for all threads available on each side of the screw. The averages of the best three consecutive-thread BMC and BA values (BMC3 and BA3) were calculated and represent the situation at the cortical passage 12 weeks after implant insertion in the nine animals.

Statistical Analysis

The Wilcoxon signed rank test was used for statistical analysis at a significance level of $p \leq .05$.

RESULTS

Topographic Evaluation

Results of the topographic analyses are summarized in Table 1. The values for the average height deviation from the mean plane (S_a) were 0.70 ± 0.1 μm and 0.78 ± 0.2 μm for the turned and blasted implants, respectively. The corresponding values for the developed surface area (S_{dr}) were 21 ± 7% and 20 ± 6%. No statistically significant differences between turned and blasted surfaces were found for these two parameters. However, a statistically significant difference was demonstrated for the spatial descriptive parameter (S_{cx}): 9.40 ± 1.7 μm for blasted surfaces versus 10.25 ± 1.5 μm for turned surfaces ($p = .009$).

Histomorphometric Evaluation

Light microscopic investigations of the ground sections revealed no obvious adverse tissue reactions around the implants, independent of surface preparations. Macrophages and multinucleated giant cells were observed in small numbers in close contact with both surface types.

The results of the morphometric analyses are summarized in Table 2. The turned implants showed a bone-to-metal contact (BMC) of 35 ± 11% whereas the blasted implants showed a BMC of 42 ± 10%. The corresponding values for the average BMC of the three best consecutive threads (BMC3) were 48 ± 15% and 52 ± 10%. The turned implants showed a bone

TABLE 3 Results of Resonance Frequency Measurements*

	Mean RF Values (kHz)	
	At Insertion	At 12 Weeks
Turned surface	6.35 ± 0.47	7.02 ± 0.56 (p = .0077)
Blasted surface	6.08 ± 0.68	7.08 ± 0.25 (p = .0117)

RF = resonance frequency.
*Mean values and standard deviations (±) for each surface modification, calculated at time of implant insertion and 12 weeks after insertion, before removal from the nine animals.

area inside the threads (BA) of 73 ± 9% whereas the blasted implants showed a BA of 70 ± 13%. The corresponding values for the average BA of the three best consecutive areas inside the threads (BA3) were 85 ± 7% and 82 ± 5%. A comparison of the variously treated surfaces revealed no statistically significant differences between BMC and BA or between BMC3 and BA3.

Resonance Frequency Evaluation

The mean resonance frequency (RF) values of the investigated implant surface preparations are summarized in Table 3. The initial stability value for turned implants was 6.35 ± 0.47 kHz; stability after 12 weeks was 7.02 ± 0.56 kHz. The corresponding values for blasted implants were 6.08 ± 0.68 kHz and 7.08 ± 0.25 kHz. For both surface preparations statistically significant differences ($p < .05$) were found between primary stability and stability 12 weeks after implantation (blasted surface, $p = .0117$; turned surface, $p = .0077$).

No statistically significant differences between turned and blasted surfaces were found either at insertion or at removal after 12 weeks.

DISCUSSION

The present study investigated and compared implant stability and bone tissue response to titanium implants prepared with isotropic and anisotropic surfaces of similar roughness. In agreement with earlier findings RF measurements demonstrated increased implant stability with time[16,17] but no statistically significant differences between the two types of surface. Also the histomorphometric results revealed no differences in bone tissue response. This corresponds well with an earlier study reported in 2001 by Hallgren and colleagues,[18] in which photolithographically prepared titanium implants with a similar roughness but different textures produced bone tissue responses similar to those produced by turned-surface controls. However, the results of the study by Ivanoff and colleagues[13] in 2001 implied that surface orientation might be as important as the degree of surface roughness since blasted microimplants showed better bone integration than turned ones did despite similar surface roughness in terms of S_a and S_{dr} values. Another explanation for Ivanoff and colleagues' results could be that the specially designed turned microimplants were very rough at the top of the threads whereas the flank areas showed normal values, resulting in average values highly exceeding what is expected for turned surfaces. If only the roughness of the flank areas had been considered, the blasted surfaces (which showed the best bone tissue integration) indeed would have shown the roughest surface. In our study the flank and top areas showed similar values.

The present study supports previous in vitro observations of the effects of geometric surface properties on cell behavior. Osteoblasts tend to attach more readily to surfaces with a rougher surface.[19] Furthermore they demonstrate decreased proliferation and increased differentiation, local factor (transforming growth factor β, prostaglandin E) protein production, and response to systemic hormones.[20–23]

From experimental in vivo studies it is clear that an increase in surface roughness may result in more bone at the implant interface, and it seems there is an optimum level of roughness above which a less strong bone response is seen.[9–11] However, a change in surface roughness may lead to a concomitant change in surface oxide thickness, that is, altered chemistry, porosity, and crystallinity, all of which are factors that cannot be overlooked when it comes to implant integration.[24–26] Changes in oxide thickness and area enlargement may also alter the rate and extent of titanium ionic leakage,[27] which would affect the cells, as titanium ions have been shown to have an inhibitory effect on calcification in vitro.[28]

CONCLUSION

The results of this study showed that titanium implants prepared with isotropic and anisotropic surfaces with similar roughness resulted in similar bone tissue responses and implant stability in rabbit bone after 3 months.

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