The Influence of Surface Roughness on the Displacement of Osteogenic Bone Particles during Placement of Titanium Screw-Type Implants

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ABSTRACT

Background: Previously, we demonstrated that bone debris, which is translocated during dental implant placement, has osteogenic potential. Therefore, it was hypothesized that implant surface roughness can influence the amount of translocated bone debris/particles and thereby the osteogenic response.

Material and Methods: Small titanium implants were left turned (smooth) or blasted and acid etched. The implants were placed in fresh cadaver bone. After explantation, the implants were incubated in a culture medium containing β-glycerophosphate and dexamethasone up to 24 days. Subsequently, histology, scanning electron microscopy (SEM), DNA analysis, and calcium (Ca) content measurements were performed.

Results: For both types of implant during implant placement, bone particles were translocated because of inherent roughness of the implant. SEM and histology confirmed the presence of a bone-like tissue on the surface of both types of implants, as also confirmed by DNA and Ca measurements. However, the significantly higher roughness of the etched implants accounted for more bone debris and accordingly elevated osteogenic response. Control samples, which had not been placed into bone, did not show mineralization in the same medium.

Conclusion: The present study, for the first time, demonstrated that implant surface roughness can increase the amount of the translocated bone particles and thereby also have a beneficial effect on the osteogenic response of these bone particles. It is hypothesized that these bone fragments behave like miniature auto-grafts and thereby play a significant role to enhance peri-implant osteogenesis. Optimization of surface topography should be evaluated to take advantage of this additional effect of surface roughness.

KEY WORDS: bone debris, in vitro, osteogenesis, surface roughness

INTRODUCTION

The placement of oral implants to treat (partially) edentulous patients has become a routine clinical procedure. Variables like surgical technique, patient bone quality, implant design, mechanical loading, and material surface characteristics influence the process of bone healing around an implant.[1] Clinical failures of smooth implants motivated researchers to develop more adequate surface characteristics.[2] To improve the surface characteristics of implants, surface treatments like grit blasting, acid etching, fluoride-modification, and calcium phosphate (Ca-P) coatings have been developed.[3] Ca-P coatings show excellent compatibility with human bone, and tend to initiate a rapid biological response, improve adhesion between bone and implant, and provide a scaffold for bone growth.[4–6] New coating techniques, like DNA-based coatings, and the addition

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of growth factors, like bone morphogenic proteins, are still in a developmental stage.[7] Therefore, until now, most research dealing with implant surface modification has been focused on roughening by means of grit blasting and/or acid etching.[8]

Implant surface roughness has been supposed to stimulate the bone cell reaction resulting in an enhanced healing response and improve implant bone contact.[9–11] Another beneficial effect of micro-roughened surface topography arises from a greater primary stability,[12,13] resulting into better bone healing and long-term survival of endosseous implants as has been suggested by several reports.[14,15]

However, besides biological and mechanical advantages of surface roughening, a third reason, why roughened implant surfaces show improved healing responses, was suggested. Histological examinations, during in vivo studies, often revealed that small bone fragments were interspersed between bone marrow spaces with clear signs of remodeling of these fragments.[16,17] Recently, it was demonstrated that such bone particles/fragments become translocated as a result of implant placement, and that these particles have an osteogenic potential.[18] This translocation of bone particles might act as a kind of miniature auto-grafting, thereby stimulating the bone healing process of the peri-implant bone.

Consequently, in the present study we hypothesized that the implant surface micro-geometry can influence the amount and thus the osteogenic response of the translocated bone. For this purpose, two types of titanium screws implants, that is, smooth (turned) and blasted subsequently acid etched, were used to analyze the influence of implant surface roughness on the amount and osteogenic response of translocated bone particles. These translocated bone particles may play a significant role in the process of new bone formation around an oral implant, thus providing a strong clinical relevance to this study.

MATERIAL AND METHODS

Implants

As a model for dental implants, 208 small diameter titanium screws (Stabilok™, Fairfax Dental Inc., London, UK) were used. All implants measured 4.5 mm in length and 0.53 mm in diameter. Two different surface topographies were used: (1) smooth ("turned"), and (2) blasted and acid-etched ("etched").

For roughening, the implants were first grit-blasted with Al_2O_3 (Corund type 20) for 30 seconds. Subsequently, the implants were submerged in acid etching solution ($HCL/H_2SO_4/H_2O$ 1:1:1) for 90 seconds at 100°C. Before use, all implants were ultrasonically cleaned in isopropyl alcohol and finally sterilized in an autoclave at 121°C for 15 minutes.

The implants were divided into experimental and control groups, for both types of implants. All groups were subdivided by incubation time. Four different incubation time points were used: 1, 6, 12, and 24 days.

UST Analysis

A universal surface tester (UST®, Innowep GmbH, Würzburg, Germany) was used to characterize the surface roughness of the titanium screw-type implants. This equipment includes a diamond stylus, consisting of a 60° cone, which is moved across a surface with a load of 1 mN and with the velocity of 0.1 mm/s. The measured surface roughness (R_a) is the arithmetic mean of the absolute values of the surface departures from a mean plane within the sampling area, measured in micrometer. For the R_a of the implants, three implants of each type were selected and measured before placement into the bone. The macro roughness was measured over a distance from 0.00 mm to 3.00 mm (ie, including several screw threads). Micro roughness was measured for the length of one thread of the implant. For each implant, three different threads were measured. The approximate distance of the selected thread was 0.18 mm.

Preparation of the Femur

The model system for implant placement was adapted from an earlier study.[18] Prior to experimentation, approval of the Radboud University Nijmegen Animal Ethics Committee was obtained. National guidelines for the care and the use of laboratory animals were obeyed. Six male Wistar rats of 40–43 days old, which were control animals from other studies, were used to isolate the femurs. Rats were sacrificed and the femurs of both hind legs were isolated free of soft tissue. A total of 12 femurs were washed three times for 15 minutes in washing medium, that is, alpha Minimal Essential Medium (α-MEM Gibco BRL, Life Technologies B.V. Breda, The Netherlands) with 0.5 mg/mL gentamycin and 3 μg/mL fungizone.

Implant Placement and Removal

After washing, the femurs were placed in sterile gauze soaked into sterile saline solution to prevent the drying of specimen. Holes were made in femurs with a sterile bur (Stabilok™ .53 mm drill) using a dental drilling device, to allow placement of the implant. Then, both implant types were manually screwed into the femur, immediately removed, and subsequently incubated in an osteogenic culture medium, that is, α-MEM, supplemented with 10% fetal calf serum (Gibco), 50 μg/mL ascorbic acid (Sigma, Chemical Co., St.Louis, MO, USA), 50 μg/mL gentamycin, 10 mM Na β-glycerophosphate (Sigma), and 10^{-8} M dexamethasone (Sigma). Incubation was performed in a humidified atmosphere of 95% air, 5% CO2 at 37°C. The medium was changed every 2 to 3 days.

Samples were retrieved after the various incubation time points. Of all implant types, also control screws, which were not placed into the bone, received the above mentioned treatment.

Total DNA Analysis

After 24 hours, 12 samples for each type were taken out for DNA analysis. Culture medium was removed and 3 implants were pooled and submerged in 200 μL MilliQ water and frozen at –20°C until analysis (i.e., resulting in $n = 4$). Cell lysates were defrosted, sonicated, and vortexed. The total DNA content was assessed by PicoGreen dsDNA Quantitation kit (Molecular Probes, Eugene, OR, USA). After brief centrifugation, to 100-μL supernatants, 100-μL PicoGreen working solution was added. After incubation, the fluorescence of each sample was measured in duplicate at 520 nm with a spectofluorometer, and DNA amounts were calculated from a standard curve.

Calcium Content

To determine the Ca-P deposition on the surface, 200 μL of 0.5 M acetic acid was added to samples collected at 1, 6, 12, and 24 days. Samples were shaken vigorously overnight to dissolve calcium (Ca) from deposited mineralized extracellular matrix. Then calcium content in the samples was measured by the ortho-cresolphthalein complexone (OCPC) method. First, 80-mg OCPC (Sigma) was added in 75-mL H_2O with 0.5-mL 1 N KOH and 0.5-mL 0.5 M acetic acid to prepare the OCPC solution. Then, working solution was prepared according to the following formula: 5-mL OCPC solution was added to 5-mL 14.8 M ethanolamine/boric acid buffer (pH = 11), 2-mL 8-hydroxyquinoline and 88-mL MilliQ water. Finally, 300-μL working solution was added to 10-μL sample or standard and measured at 570 nm with a spectofluorometer.

Scanning Electron Microscopy (SEM)

Duplicate samples from days 1, 6, 12, and 24 were rinsed twice with PBS, fixed in 2% glutaraldehyde for 5 min, and subsequently washed with 0.1 M Na-cocadylate buffer. Thereafter, samples were dehydrated in a graded series of ethanol, dried with tetramethylsilane, and kept dry until SEM evaluation. Just before analysis, gold was sputtered on the specimens. The specimens were examined and recorded using SEM microscopy.

Histological Procedures

The specimens for histology were fixed in formaldehyde 4%, dehydrated in ethanol, and embedded (non-decalcified) in methylmethacrylate (MMA). After polymerization of the MMA, thin (10 μm) non-decalcified sections were prepared with a modified diamond blade sawing microtome technique.[19] Three sections were made in a transversal direction perpendicular to the axis of the implant. The sections were stained with methylene blue/basic fuchsin and examined with a light microscope to confirm the presence of bone-like tissue on the surface of implants.

Statistical Analysis

To ensure the reproducibility, two separate runs of the experiment were performed which gave near identical results. All data as presented are from one experiment, and is expressed as mean ± standard deviation. Means between the two groups were compared by the use of a Student's *t*-test. All calculations were performed with the GraphPad® Instat 3.05 software (GraphPad Software Inc., San Diego, CA, USA). Differences were considered as significant when $p < .05$.

RESULTS

Surface Characterization

UST Analysis. Surface topographic evaluation demonstrated that both experimental surfaces differed in surface roughness (Table 1). The turned implants showed an average surface macro- and micro-roughness significantly lower than that of the etched implants.

TABLE 1 The Mean ± Standard Deviation Value of Surface Roughness (R_a µm) Measurements of Both Types of Titanium Screw-Type Implants. Macro Roughness was Measured over a Distance of 3 mm, whereas Micro Roughness was Measured over a Single Thread Distance of 0.18 mm

	Turned	Etched	*p* Value
Macro roughness R_a (µm) distance (0.00–3.00 mm)	1.330 ± 0.09	2.420 ± 0.29	$p < .05$
Micro roughness R_a (µm) distance (0.00–0.18 mm)	1.141 ± 0.21	1.635 ± 0.59	$p < .05$

SEM. The surface morphology was also examined by SEM for each type of implant. The SEM micrographs clearly show two different surface topographies (Figure 1). Turned implants showed a relatively smooth surface typical for titanium whereas some fabrication artifacts were observed, usually adjacent to screw threads. The grit blasted and acid-etched surface showed a uniformly roughened surface.

Total DNA Analysis

Total DNA analysis is presented in Figure 2. On day 1, significantly higher amounts of DNA were found for the etched implants as compared with the turned ones.

Calcium Content

Calcium content for turned and etched implants was measured as an indication of matrix mineralization and is depicted in Figure 3. The amount of calcium was increased over time, and was found to be significantly higher for etched implants compared with the turned implants on days 1 and 6. No significant difference was found at the later time points between the two groups. For control samples, which had not been inserted into bone, no calcium content could be measured even after incubation for 24 days in the same calcium and phosphate containing medium.

SEM

The results of the SEM analysis are depicted in Figures 4–6. SEM of experimental screw-type implants from days 1, 6, 12, and 24 revealed the presence of a bone-like tissue on their surface (Figure 4). On day 1, bone-like tissue was seen on both types of implants as the result of immediate implant removal after placement into the bone. Compared with the turned implants, the etched implants evidently showed a higher amount of bone like tissue. On the turned implants, the bone-like tissue was only present directly adjacent to the screw threads. In contrast, the etched implants had bone-like tissue adhering over almost their entire surfaces (Figure 5). With incubation time from day 1 to day 24, an increase of tissue formation was visible on both types of implants. On day 6, when compared with the turned implants, still more bone-like tissue was present on the etched implants. On day 24, upon visual inspection, no difference could be observed anymore (Figure 6). The

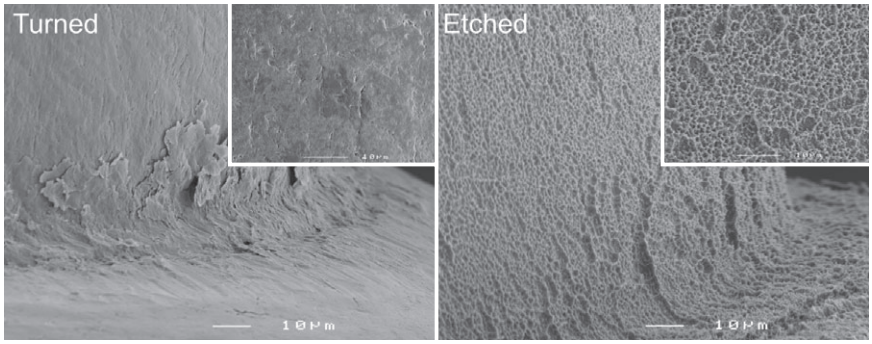

Figure 1 Surface of the turned and etched titanium screw-type implants visualized by scanning electron microscopy. Magnification for all images is 1000×, inbox image magnification 2500×.

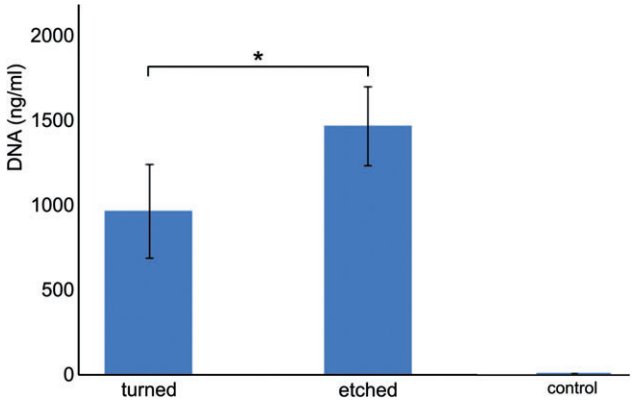

Figure 2 Total DNA on day 1; note that levels are significantly higher for etched as compared with turned implants. DNA measurements for all control groups were zero.

control samples, which had not been placed into the bone, did not show the build-up of mineralized material on their surface, even after 24 days of incubation into same phosphate-rich culture medium.

Histological Evaluation

Histological evaluation also confirmed the presence of bone-like tissue on both types of implants (Figure 7). With increased incubation time from day 1 to day 24, an increase of tissue formation was observed on all implants. However, more bone-like tissue was seen on the etched compared with the turned implants. At higher magnification, the bone-like tissue was characterized by osteoblasts and osteocytes embedded into an extracellular matrix. Furthermore, a noticeable difference existed between the old bone particles which stained a darker red compared with the newly formed extracellular matrix, thus confirming that the deposited material was a bone-like tissue, not just a mineralized matter.

DISCUSSION

In the present study, we investigated the effect of surface roughness on the amount and osteogenic response of bone particles that are translocated onto the surface of dental implants during their placement into the bone. Our findings (SEM, DNA analysis, calcium content, and histology) confirmed that the amount and subsequently osteogenic response of these translocted bone particles was significantly higher on etched as compared with turned (smooth) implant surfaces.

The validity of the animal model used in the present study has been confirmed in our previous study.[18] However, some modifications were executed. Most noticeable is that herein an osteogenic culture medium containing dexamethasone was used to evaluate the osteogenic response of the translocated bone particles. In the present study, we confirmed the presence of bone-like tissue after one day of incubation by SEM micrographs. Cell activity was also evident by the presence of high amount of DNA at early time points. The amount of DNA present at day 1 was significantly higher for etched as compared with turned implants. This is in accordance with our SEM findings. However, a DNA decline on later time points was observed (data not shown). As reported before, this is explained by impaired DNA retrieval due to the mineralization.[18,20,21] Thus, we chose not to present DNA data after day 1.

The normal range of R_a for turned implants is from 0.45 µm[11] to 0.7 µm.[22] Turned implants used in the present study have R_a value of 1.14 (±0.21) µm. Diversity exists in measurement methods for evaluating surface profile of dental implants. Therefore, precise comparison in various studies is complicated.[23] However, in recently performed systematic review, wide range of surface roughness ranging from R_a/S_a 0.5 µm up to 8.5 µm have been suggested which can positively influence the bone response around the implant.[23]

The SEM micrographs and Ca measurements demonstrated the deposition of mineralized/calcified tissue with a gradual increase in amount from day 1 to day 24,

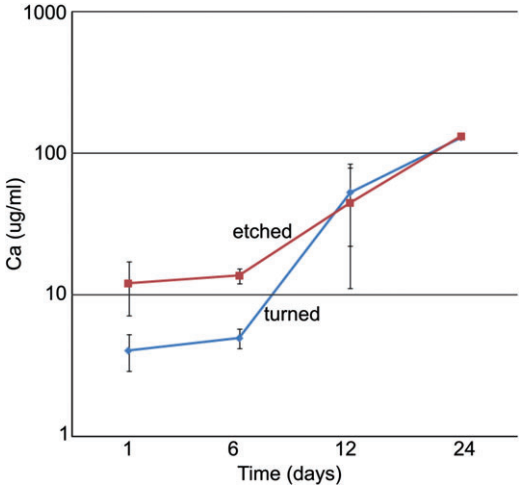

Figure 3 Calcium content measurements on days 1, 6, 12, and 24. On day 1 and day 6, the etched implants have significantly higher calcium present on their surface as compared with the turned implants (note the logarithmic scale). Calcium measurements for all control groups were zero and because of the presence of logarithmic scale could not be shown in the figure.

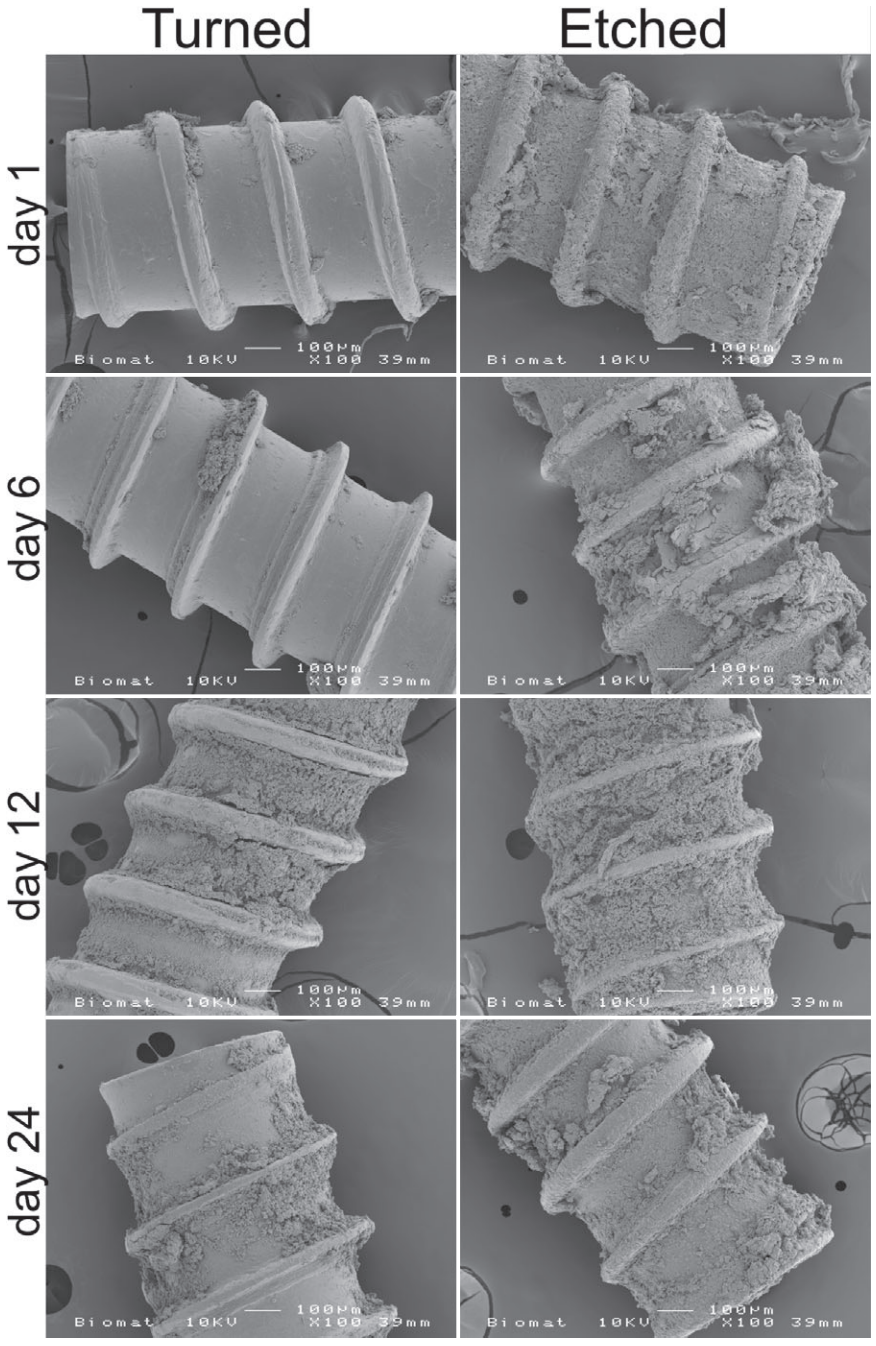

Figure 4 Surface of experimental groups (turned and etched) visualized by scanning electron microscopy. Magnification of all micrographs is 100×. Note that on all types of implants, a bone-like tissue was observed on day 1. The gradual increase in deposition of tissue was seen from day 1 to day 24. Etched implants contained more bone-like tissue on early time points as compared with the turned implants, but no visual difference was observed on later time points.

on the turned as well as on the etched implants. Many studies have confirmed the differentiation of osteoblast-like cells in the presence of dexamethsone. Moreover, formation of calcified tissue has been observed after 12 to 14 days of incubation of such cells in the same culture medium.[21,24] We therefore concluded that translocated bone particles indeed played a major role in the further development and mineralization of bone-like tissue on implants.

Osseointegration is a dynamic process during its establishment as well as its maintenance.[25] The effects of implant roughening are essential to understand, as

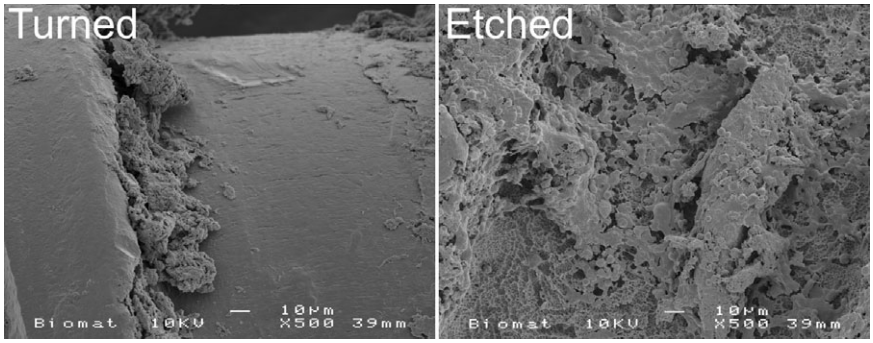

Figure 5 Surface of experimental groups (turned and etched) on day 1. Magnification of micrographs is 500×. Note that on the turned implants, the bone-like tissue is only adherent to screw threads, in contrast etched implants contained more and more evenly distributed bone-like tissue.

clinical results show that surface-roughened implants have a five times lower failure rate (3.2%) compared with turned implants (15.2%).[26] The implant surface topography can influence the rate and extent of osseointegration, which is generally expressed by the amount of bone-to-implant contact (BIC).[27] For example, in poor bone quality sites, implants with an acid-etched surface (Osseotite®, Implant Innovations, Inc., Palm Beach Gardens, FL, USA; OSS group) can achieve a significantly higher BIC as compared with implants with a turned surface.[28,29]

In recent years, many in vivo and in vitro studies have been performed to understand the exact mechanism behind superior healing response of roughened implants. Several studies demonstrated that rough surfaces provide a favorable environment for attachment,[30,31] differentiation, and proliferation[32–34] of osteoblast-like cells and other biological activities (e.g., release of growth factors)[35] involved in the bone healing process. Rough surfaces are considered "osteophilic".[26] In the present study, we presented a new aspect which might also be contributing to the known beneficial effects of surface roughness. In clinical practice, the placement of implants with rough surfaces are capable of loosening more bone particles as compared with turned surfaces. These particles by themselves function

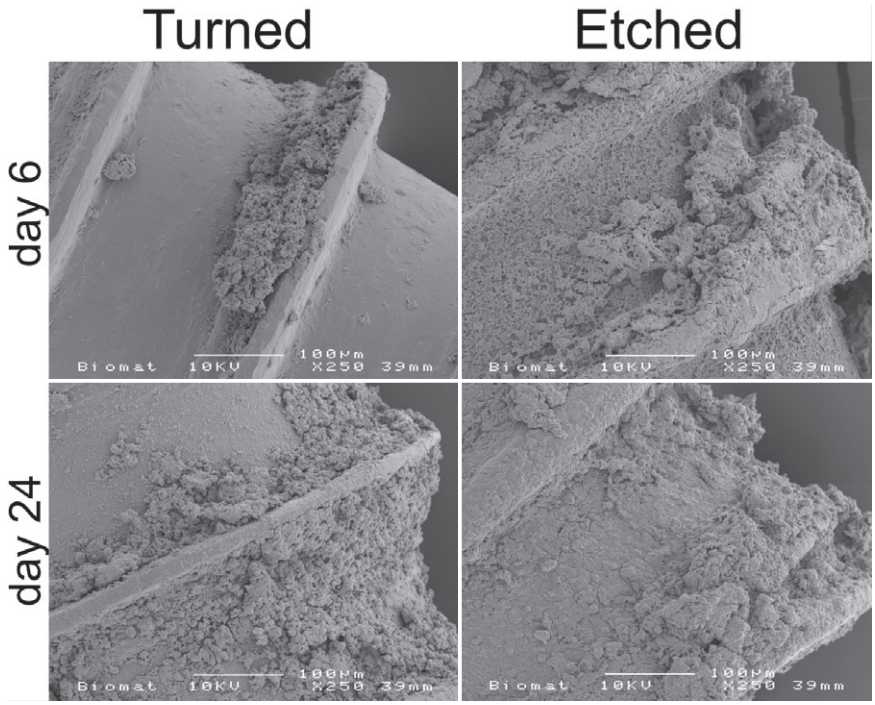

Figure 6 Surface of experimental groups (turned and etched) visualized by scanning electron microscopy on day 6 compared with day 24. Magnification of all figures is 250×.

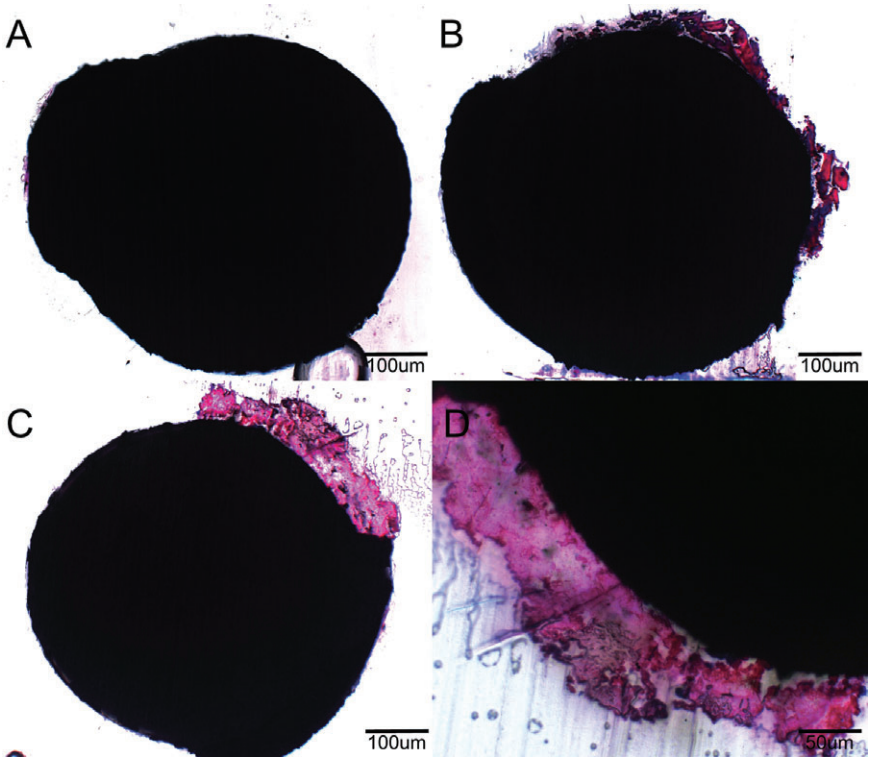

Figure 7 Light micrographs of histological sections showing bone-like tissue on the surface of the implants. In Figure 7*A* (turned) and 7*B* (etched) implants can be seen at day 6. In Figure 7*C*, turned implants at day 24 are shown. Magnification of all figures from 7*A–C* is 10×. In Figure 7*D*, higher magnification of turned implants at day 24 is shown (magnification 40×).

as micro bone transplants. Our hypothesis is supported by a recently performed animal study which confirmed that loosened host bone fragments/particles became enveloped in a newly formed peri-implant trabecular bone and there favored osteogenesis.[36]

In the present study, for the first time, we clearly demonstrated that implant surface roughness can increase the amount of the translocated bone particles and thereby also have a beneficial effect on the osteogenic response. It can be hypothesized that these bone fragments in the clinical situation behave like a miniature auto-graft and may play a significant role to enhance peri-implant osteogenesis. However, further in vivo studies should be performed to better understand the role of translocated bone particles in the process of new bone formation. Also optimization of surface topography should be evaluated to take advantage of this additional effect of surface roughness.

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