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Surface-conditioned dental implants: an animal study on bone formation

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Abstract

Aim: The aim of this study was to determine whether bone formation around surface-conditioned implants is enhanced compared with non-surface-conditioned sandblasted acid-etched titanium implants.

Materials and Methods: One hundred and forty-four implants were placed in the mandible of 18 minipigs. Before placement, implants were either surface conditioned in a solution containing hydroxide ions (conSF) or assigned to controls. Animals were euthanized after 2, 4 and 8 weeks of submerged healing, the 8-week group receiving polyfluorochrome labelling at week 2, 4, 6 and 8. One jaw quadrant per animal was selected for histological and histomorphometrical evaluation of mineralized bone–implant contact (mBIC), osteoid–implant contact (OIC) and bone volume (BV) analysis.

Results: Polyfluorochrome labelling showed no general differences in bone dynamics. mBIC showed the most pronounced differences after 2 weeks, reaching 65.5% for conSF compared with 48.1% for controls, $p = 0.270$. Differences levelled out after 4 weeks (67.4% control, 65.7% conSF) and 8 weeks (64.0% control, 70.2% conSF). OIC levels were initially comparable, showing a slower decline for conSF after 4 weeks. BV was higher for conSF at all times. No significant differences could be found.

Conclusion: A tendency towards increased mBIC was shown for surface-conditioned implants after short-term healing.

Key words: conditioning; dental implant; histomorphometry; osseointegration

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A major focus of research in dental implantology has been on the development of new implant surface conditions. Surface characteristics are determined by factors like topography, chemical properties, surface charge and wettability (Albrektsson & Wennerberg 2004). Among others, chemically modified implants have been reported to promote the early stages of bone apposition (Buser et al. 2004).

The modification of implant surface topography has been shown to affect the rate of bone formation and biomechanical fixation (Cochran et al. 1998). It is the influence of modified macro- and microroughness that is of essence. Most commonly applied techniques to influence surface microroughness are subtractive processes that alter the surface texture (Ellingsen 1999). Techniques like dual acid etching or sandblasting and acid etching showed advantageous bone formation in comparison to controls (Cochran et al. 1998, Veis et al. 2004). The microscopic features of textured surfaces are considered to interact with peri-implant cells, which may have implications in terms of the mechanisms by which these surfaces influence tissue formation (Simmons & Pilliar 1999). Microtopography was also described to influence the proliferation and differentiation of osteoblastic cells (Brunette 1988).

At the same time, the interaction of an implant surface and the surrounding natural environment in bone is not solely influenced by surface topography. There is a considerable body of

Conflict of interest and source of funding statement

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evidence showing that surface chemistry influences the early stages of bone formation. An enhanced surface energy and wettability have been demonstrated to stimulate the interaction between the implant surface and its biological environment (Baier et al. 1984). The amount of wettability can be determined by contact angle measurements, providing ranges from 0° (hydrophilic) to 140° (hydrophobic) for titanium surfaces (Le Guéhennec et al. 2007). Hydroxylated/hydrated surfaces were shown to have immediate wettability, leading to more differentiated osteoblast phenotypes and to yield higher local factors (Zhao et al. 2005). Such an effect might lead to enhanced bone formation and consecutive osseointegration.

Surface conditioning by hydroxide ions increases the surface energy of titanium implants. This reduces the contact angle below 5°, implying a high degree of wettability. Such a surface treatment enables further fast and homogenous protein absorption.

The aim of this study was to determine whether bone formation around surface-conditioned implants is enhanced compared with non-surface-conditioned sandblasted thermally acid-etched titanium implants. Surface-conditioned implants were identical to control implants, but were dipped in a hydroxide ion solution before implantation. The hypothesis was that bone formation is increased compared with non-surface-conditioned implants.

Materials and Methods

Animals

Eighteen females, 18-month-old Mini Lewe miniature pigs, approximate weight 50 kg, were used. Animal selection and surgical protocol were approved by the commission for animal studies at the district government office Dresden, Germany. The animals received a soft diet and had free access to water. The oral cavity was cleaned before surgery.

Implants

Threaded titanium implants (SPI® Element, Ø3.5 mm × 9.5 mm, Thommen Medical AG, Waldenburg, Switzerland) with a sandblasted and thermally acid-etched surface were applied. The sterile implants were surface conditioned with hydroxide ions (conSF) or left untreated (stanSF). The source of the hydroxide ions was a sterile diluted sodium hydroxide solution. The concentration of the solution was 0.05 M. Sterile implants were unpacked; each implant was dipped in a test tube containing the sterile conditioning solution. During the dipping process, the test tube was placed in a water bath under ultrasound application. The implant was incubated for 20 s at room temperature. After removing the implant from the test tube, implantation was performed immediately.

Surgical procedure

For anaesthesia, a solution of midazolam (1 mg/kg i.m., Ratiopharm GmbH, Ulm, Germany) and ketamine (10 mg/kg i.m., Riemser Arzneimittel AG, Greifswald, Germany) was applied. To reduce salivation, atropine (0.05 mg/kg) was added to the injection. After surgery, carprofen (2–4 mg/kg SC, Rimadyl©, Pfizer Pharma GmbH, Berlin, Germany) was administered.

The mandibular primary pre-molar teeth were surgically extracted. After a healing interval of 9 weeks, the permanent mandibular pre-molar teeth were extracted. Care was taken to avoid the fracture of bone walls. The extractions were performed under general anaesthesia and local dental infiltration anaesthesia of lidocain (2 ml, Xylocitin© 1% epinephrine, Mibe GmbH, Brehna, Germany).

After a 9-week healing interval, titanium implants were placed in the edentulated mandibular alveolar ridge under amoxicillin (15 mg/kg i.m., Duphamox©, Fort Dodge Vet. GmbH, Würselen, Germany). Anaesthesia was performed as described earlier. A mucoperiosteal flap was elevated, performing an incision along the vestibular region and two releasing incisions in the perpendicular direction over the alveolar crest. Any remaining sharp bone crests were flattened, using a surgical drill under permanent water cooling. Each miniature pig was scheduled to receive four implants (two surface-conditioned implants and two control implants/side) in each side of the mandible. This amounted to eight implants per pig. One mandibular side was allocated to histology, the other side to removal torque testing, which will be reported in a separate publication. The surgeon was blinded in terms of side allocation. Implant positions were alternated, being determined using random permuted blocks. The implants were endosseously placed according to the surgical protocol of the manufacturer. Inter-implant distance was 3 mm. Placement was performed by a maxillofacial surgeon (R. M.), experienced in implantology and animal surgery. Following implant placement, a cover screw was placed on the implant and the flap was repositioned using resorbable sutures (PGA Resorba 4 × 0®, Resorba, Nürnberg, Germany).

Intra-surgery photographs were taken during implant conditioning and placement (Figs 1 and 2).

Polyfluorochrome labelling

To visualize the dynamics of bone formation, sequential polyfluorochrome labelling was performed for the 8-week healing group at 2, 4, 6 and 8 weeks after implantation. These six pigs were given tetracycline (20 mg/kg i.v., Doxycylin©, Ratiopharm GmbH) 2 weeks after implantation. Alizarin complexone (30 mg/kg i.v., Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) was administered after 4 weeks, followed by calcein green (20 mg/kg i.v., Sigma-Aldrich) after 6 weeks. Finally, xylenol orange (90 mg/kg i.v., Sigma-Aldrich) was given after 8 weeks, 2 days before euthanasia. All these procedures were performed under general anaesthesia.

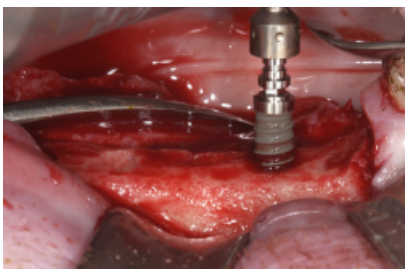


Fig. 1. Implant placement – conditioned surfaced implant.

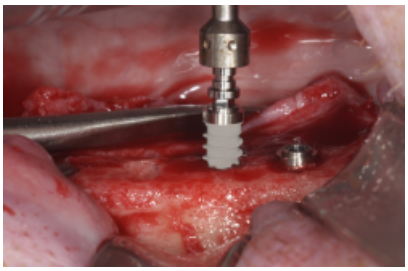


Fig. 2. Implant placement – control implant.

Post-surgical procedure

The pigs were euthanized in three groups of six animals each after 2, 4 and 8 weeks of healing by an overdose of T 61© (10–15 ml i.v., 200 mg/ml embutramid, 50 mg/ml mebezonium iodide/5 mg/ml tetracain, Intervet Deutschland GmbH, Unterschleissheim, Germany) under anaesthesia. After euthanasia, the mandibles were resected and separated into two halves. Following a predetermined statistical protocol, one side was subjected to histology, and the other side to removal torque testing.

Immediately post euthanasia, the mandible sides allocated to histology were radiographed (Orthophos CD®, Sirona, Bensheim, Germany) to localize the implants. Further, digital volume tomography (Accuitomo©, J. Morita Corp., Kyoto, Japan) was performed.

Following radiography, mandibular en bloc sections including the implants, alveolar bone and the mucosa were collected, rinsed and transferred to 10% neutral-buffered formalin.

Histological procedure

The mandibular samples were fixed in formalin and dehydrated in a graded series of ethanol. Next, the implants with surrounding bone were embedded in methylmethacrylate (Technovit 9100 neu®, Heraeus Kulzer, Wehrheim, Germany). Each implant region was dissected using a diamond saw (Exakt-Apparatebau, Norderstedt, Germany). Undecalcified 200-μm-thick sections along the implant length axis in the bucco-oral direction were cut using a diamond saw microsectioning system (Isomet 1000®, Buehler GmbH, Düsseldorf, Germany). Thus, approximately three to four sections could be gained per implant.

Thereafter, micrographs of the middle section of each implant were taken at this thickness, using a high-resolution analogue film (Kodak Oncology Film, Eastman Kodak Company, Rochester, NY, USA). This was performed to visualize the stage of calcification of the bone samples adjacent to the titanium implants.

Following microradiography, the sections were reduced to 30 μm in thickness using Donath's grinding techniques (Donath & Breuner 1982) on a roll grinder containing sandpaper (Exakt-Apparatebau). Subsequently, fluorochrome microscopy was performed for the 8-week group. Upon completion, all histological sections were stained according to Masson–Goldner.

Data analysis

Radiographs and micrographs were qualitatively evaluated with regard to placement position and peri-implant bone formation by one masked experienced examiner (B. S.).

Fluorescence microscopy was performed at up to $\times$ 40 magnifications (Olympus Optical GmbH BX 61, Hamburg, Germany) by two masked examiners (B. S., R. M.). Polyflurochrome labels were qualitatively analysed for bone growth dynamics, location and order of the labels and secondary remodelling.

The histologic and histomorphometric analyses were performed using light microscopy (Olympus Optical GmbH BX 61). Histology was analysed by two masked examiners (B. S., R. M.) at up to $\times$ 20 magnification. The observation focused on the implant threads and the neighbouring bone. This area was evaluated for bone formation, osteoid reaction, woven and lamellar bone, inflammatory response and bone remodelling.

In order to perform histomorphometry, the sections were imaged by a digital camera (Colour View 2, Olympus Optical GmbH) at $\times$ 4 magnification, using a motorized measuring stage (Märzhäuser, Wetzlar, Germany) for multiple alignment scanning connected to a computerized system of histomorphometry (Analysis, Soft Imaging Systems, Münster, Germany). Histomorphometric measurements were performed by one masked, calibrated examiner (A. T. L.). All histometric measurements were performed in three neighbouring implant threads. Counting from coronal, the region of interest started at the third implant thread, being located 2 mm below the implant neck. The region of interest is depicted in Fig. 3. (Fig. 3) the following parameters were determined:

Mineralized bone–implant contact (mBIC) was measured along the three implant threads. Osteoid–implant contact (OIC) was equally determined along the region of interest, yielding the percentage of osteoid in contact with the implant surface.

Bone volume (BV) analysed the percentage of bone matrix (mineralized and unmineralized bone) excluding marrow islands or soft tissue (Parfitt et al. 1987). A tangent line was placed at the tips of the three implant threads mentioned, defining the area of measurement within the threads (Fig. 4).

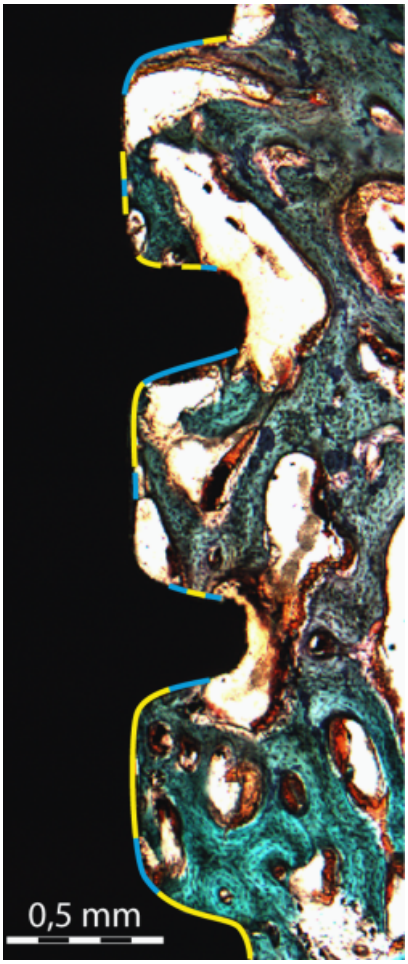


Fig. 3. Region of measurement for mineralized bone–implant contact (yellow) and osteoid–implant contact (blue) along three implant threads.

The percentages of mBIC, OIC and BV were determined for every histological section. Mean values were calculated for each implant and for each group of implant surface state.

Statistical analysis

A non-parametric statistical approach was chosen. The Mann–Whitney and the Kruskal–Wallis test were applied to analyse the effects of surface conditioning and time. Because of multiple comparisons, the significance level was adjusted according to the Bonferroni procedure. Statistical analysis was performed by SPSS for Windows® 15.0.1 (SPSS Inc., Chicago, IL, USA) and by SAS for Windows® 9.2 (SAS Institute Inc., Cary, NC, USA). Data are illu-

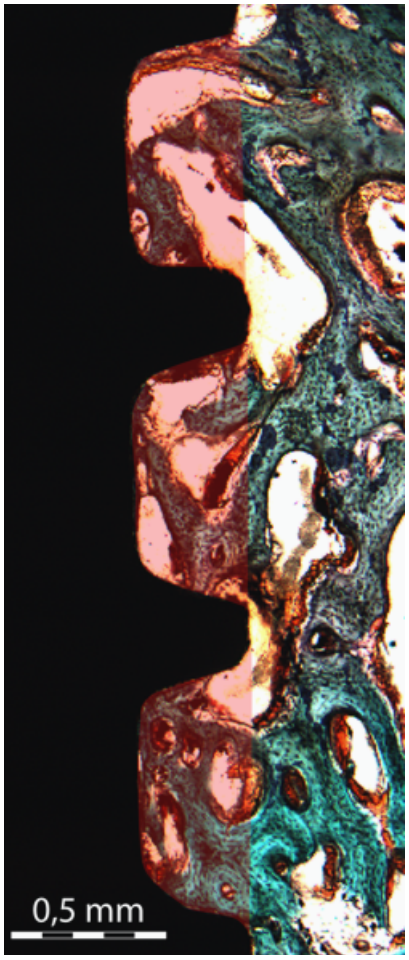


Fig. 4. Area of measurement (red) for bone volume along three implant threads.

strated by box plots. Median values with upper and lower bounds are shown.

Results

Post-surgical observation

One animal assigned to the 2-week group developed an abscessus in the operated area and was excluded from the evaluation. At euthanasia, cover-screws were in part exposed for most implants without clinical signs of inflammation. One conSF implant assigned to the 2-week groups was clinically found to be mobile at the time of euthanasia and considered as implant loss.

Radiographic/micrographic observation

At euthanasia, digital volume tomography revealed the three-dimensional implant positions with the mandible. All implants were placed well centred without major lateral contact to cortical bone.

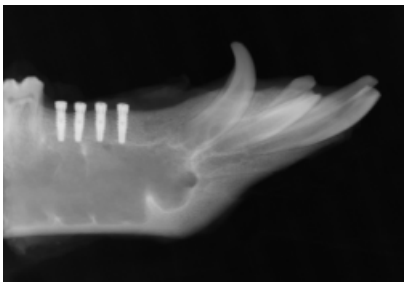


Fig. 5. Dental radiography 4 weeks after implantation. Implant surface states from left to right: conditioned, control, conditioned and control. No major differences could be detected between the surface states.

Dental radiography showed no differences between control and the surface-conditioned implants. In some cases, crestal bone loss was observed for implants with exposed cover screws (Fig. 5).

Microradiography visualized the implant cross section along the bucco-oral axis. The evaluation showed detailed radiodense structures, representing mineralized bone. This visualized the peri-implant bone structure and matched the histological sections. No additional quantification of micrographs, next to histomorphometry was performed (Fig. 6).

Fluorescence microscopy

Polyfluorochrome labelling showed newly formed bone on the implant surfaces and the host bone. While alizarin (red) and calcein green labels were clearly distinguishable, the tetracycline (yellow) and xylenol orange labels were scarcely detectable. Demonstrating the timely manner of bone formation, alizarin labels were observed closer to the implant surface and deeper in the osteons of the host bone, followed by adjacent calcein labels. This suggests the mentioned new bone formation on the implant surface before the fourth week. Bone formation must have started from both the implant surfaces and then lamellar bone formation continued as visualized by the consecutive alizarin and calcein fluorochrome labels. Further, ''kissing bone contacts'' at the tips of the threads suggest bone formation starting from the peri-implant bone. The resorption of some alizarin and calcein labels indicated processes of secondary remodelling after the sixth week post implantation (Fig. 7).

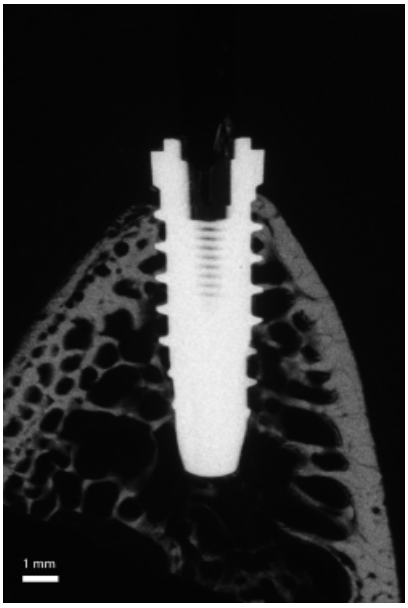


Fig. 6. Microradiography 8 weeks after implantation. Calcified peri-implant bone structure around a conditioned surfaced implant.

Histomorphology

Implants exhibiting exposed cover screws showed some resorption at the level of crestal bone. There were no indications of inflammatory processes at the deeper implant interfaces.

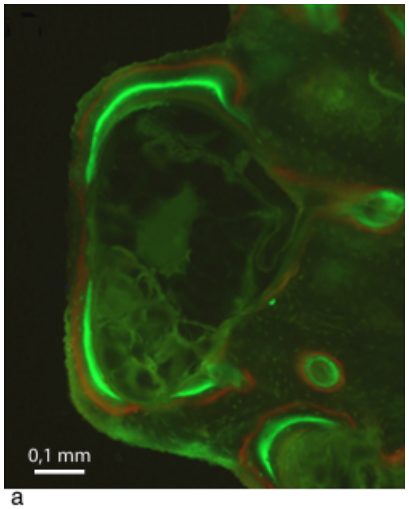
2-week healing

After 2 weeks, formations of woven bone were present within the implant threads. On the one hand, a continuous spreading of new bone on the implant surfaces could be observed that had not yet reached the inner thread areas. On the other hand, this new bone formation originated from punctual contacts with peri-implant host bone, being referred to as ''kissing spots'' that could be observed at the outer thread areas. Also within the host bone osteoid with osteoblast seams indicated active new bone formation.

At this time, both surface states exhibited the described characteristics. However, the surface-conditioned implants showed a higher amount of mineralized bone area within the threads.

4-week healing

The implant surfaces within the implant threads are partly filled by more mineralized bone with little osteoid, compared



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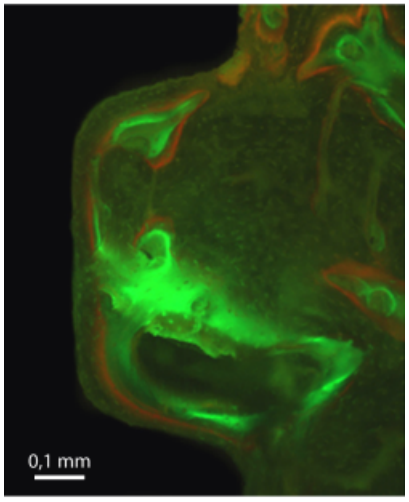

b

Fig. 7. Fluorescence microscopy 8 weeks after implantation with clearly detectable alizarin (red) and calcein (green) labels. (a) Control implant, (b) surface-conditioned implant (magnification × 10).

with the 2-week observation. The amount of osteoid also declined within host bone. New bone formation was triggered by continuous spreading of bone on the threads, therefore gaining in thickness for both surfaces. Osteogenesis was now directed from the implant surface towards the host bone as supported by fluorescence labels. Mineralization fronts were still clearly detectable after 4 weeks of healing. Newly formed bone showed cement lines separating singular lamellae, indicating a more mature stage of bone formation compared with the 2-week period.

In comparison with control implants, a higher degree of osteoid and multiple mineralization fronts could be observed around surface-conditioned implants.

8-week healing

After 8 weeks, peri-implant structural composition was comparable to the 4-week period for both surfaces. Implant threads were almost completely filled with mineralized bone. The percentage of osteoid further declined, disappearing from the implant surface. Further bone formation was limited to the intermediate zone between the threads and the host bone. Comparing the two surface states, no differences could be found in the degree of maturity of bone or remodelling processes. At this time point, both surfaces were almost completely surrounded by lamellar bone (Fig. 8).

Histomorphometry

2-week healing

After 2 weeks, an mBIC of control implants of 48.1% was found. The mBIC of conSF implants reached 65.5%. This difference was statistically not significant ($p = 0.270$).

The OIC of control implants reached 18.7%, which also exhibited no significant difference from conSF implants (20.8%, $p = 0.965$).

BV of mineralized and unmineralized bone within the implant threads was 61.4% for control implants. BV of the conSF implant was 69.8%, showing no significant difference ($p = 0.289$).

4-week healing

After 4 weeks, the mBIC values increased for control implants to 67.4%. The mBIC of conSF implants was 65.7%. The difference between the two groups was not significant ($p = 0.712$). The increase of mBIC from 2 to 4 weeks of healing was not significant for control ($p = 0.087$) and conSF implants ($p = 0.394$).

Control implants exhibited a lower OIC of 11.2% compared with 15.8% for conSF implants. The difference was not significant ($p = 0.389$). The lower OIC values compared with the 2-week period were not significant for control ($p = 0.102$) and conSF implants ($p = 0.356$).

BV of control implants was 53.7% compared with conSF implants with 65.1%, being statistically significant ($p = 0.049$). The lower values compared with the 2-week values were not significant for control ($p = 0.470$) and for conSF implants ($p = 0.851$).

8-week healing

After 8 weeks, control implants attained an mBIC of 64.0%. The mBIC of conSF implants was 70.2%. This difference was not significant ($p = 0.538$). The decrease in mBIC for control implants from 4 to 8 weeks ($p = 0.951$) and the increase for conSF ($p = 0.902$) were not significant.

OIC reached 7.3% for control implants and 7.9% for conSF implants, showing no significant difference ($p = 0.498$). The decrease in OIC from 4 to 8 weeks was significant for control implants ($p = 0.027$), but not significant for conSF ($p = 0.085$) implants.

BV of control implants was 43.6% compared with conSF implants with 53.9%, showing no significant difference ($p = 0.538$). The lower values compared with the 4-week values were not significant for control ($p = 0.622$) and conSF implants ($p = 0.065$) (Table 1, Figs 9–11).

Discussion

The aim of this study was to determine whether surface conditioning of sandblasted acid-etched titanium implants would enhance bone formation compared with controls. Test implants were surface conditioned before implant placement. Implants were placed in the mandible of 18 minipigs. The animals were euthanized after 2, 4 and 8 weeks.

At placement, the hydrophilic nature of the surface could be observed by the immediate attachment of blood and wound fluid to the implant surface. Radiography and microradiography exhibited firm bone anchorage at all healing periods for both surface states.

The results obtained from this study revealed information about the quantity and the dynamics of bone formation around surface-conditioned implants. The comparison of histomorphology between the two surfaces did not show major differences in the bony structures. A continuous spreading of new bone on the implant surfaces was observed, which is characteristic for threads that are in close contact with host bone, offering a short distance to be covered for immigrating osteoblasts. The osteogenic potential of both surfaces was pronounced. Analysing mBIC values, surface-conditioned implants showed an increased mBIC level after 2 weeks compared with controls. This difference levelled out after 4 and 8 weeks. The

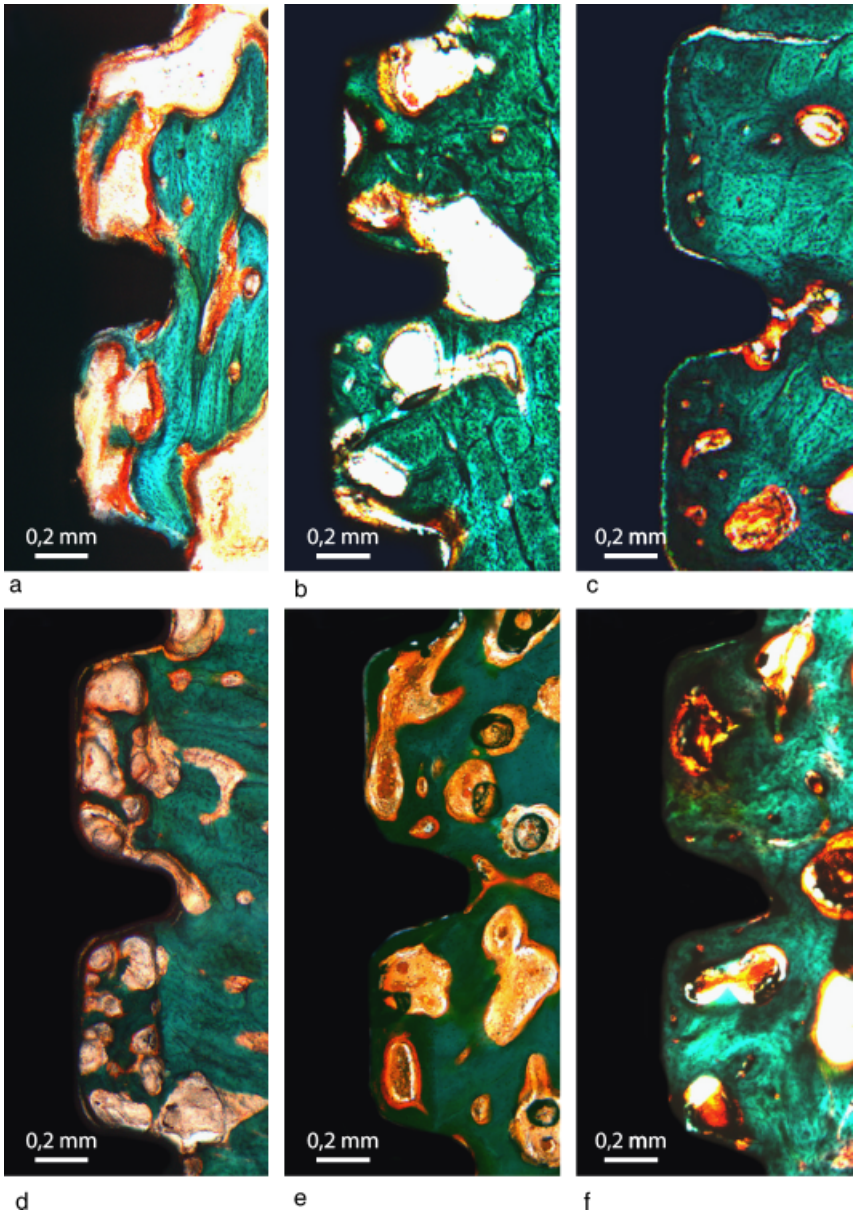

Fig. 8. Light microscopic images of control (top row) and conditioned (bottom row) surfaced implants 2 (left), 4 (middle) and 8 (right) weeks after implantation (magnification × 4, Masson–Goldner stain). (a) Originating from ''kissing host bone contacts'', some woven bone formations with abundant red-stained osteoid seams can be seen in the threads. (b) The threads appear to be partly filled by more mineralized bone with little areas of osteoid. About half of the implant surface is covered by bone. (c) Implants threads appear to be almost entirely filled by mineralized bone with a high degree of maturity. The tip of the implant thread shows secondary remodelling. (d) Direct bone–implant contact (BIC) established by ''kissing bone contacts''. More woven bone trabeculae with osteoid seams are visible within the two threads. (e) Most of the implant surface is covered by bone. The surfaces of the vascular channels within the newly formed bone in the threads and in the host bone are covered by red-stained osteoid seams. (f) Almost entire BIC, provided by dense filling of the threads by mature bone.

dynamics of new bone formation is represented by 19% (control) and 21% (conditioned) of osteoid at the implant surface. These comparable initial OIC levels showed a slower decline for surface-conditioned implants after 4 and 8 weeks. BV was higher for conditioned surfaces at all times. The main differences mentioned between the surface states did not reach statistical significance.

Comparing the results with the literature, the finding that BIC after 2 weeks was 66% for surface-conditioned implants compared with 48% for controls is in accordance with data reported previously. Schwarz et al. (2007) performed a pilot study in 4 dogs. BIC measurement determined an approximate BIC of 68% for hydrophilic implants and 54% for controls after 2 weeks. However, these values were determined from the mean values of upper and lower jaw implantations, limiting their comparability.

In a recent study, Bornstein et al. (2008) implanted commercial hydrophilic implants into mandibles of five dogs. BIC of newly formed bone was evaluated. Hydrophilic implants resulted in a significant increase in BIC, yielding 28% compared with 22% for controls after 2 weeks. This difference equally diminished after 4 weeks, yielding a BIC of 38% for both surface states. The dynamic patterns of bone formation seem to match our results. However, the lower absolute values can be attributed to different factors like sample size and animal model. Bornstein and colleagues describe comparable histological structures around hydrophilic and control implants, observing newly formed bone trabeculae, extending from the host bone towards the implant surface after 2 weeks. Our histologic findings are in agreement.

Buser et al. (2004) inserted hydrophilic experimental implants into the anterior maxilla of six minipigs. After 2 and 4 weeks, BIC was significantly increased in comparison with controls. BIC values after 2 weeks were 49% for hydrophilic surfaces compared with 29% for controls. A scaffold of woven bone formation is described. After 4 weeks, reinforced woven bone trabeculae and a deposition of parallel-fibred bone were observed. BIC was 82% for hydrophilic surfaces, compared with 67% for controls. This difference diminished after 8 weeks. Although a strict comparison between the studies cannot be made due to implicit differences in implant location, implant design and animal model, Buser and colleagues demonstrate related dynamics of bone formation by an early effect that levels out at later times.

In general, the comparison of histometric values of these different studies with our results shows a similar trend of absolute values and supports the hypothesis that more bone formation will be found around hydrophilic implants mainly during the early healing phase.

Table 1. Results from the histomorphometric measurements of control and surface-conditioned

	mBIC (%)	Lower bound	Upper bound	OIC (%)	Lower bound	Upper bound	BV (%)	Lower bound	Upper bound
2 weeks									
Control	48.05	22.14	78.95	18.65	4.95	45.05	61.36	25.66	76.74
Conditioned	65.49	32.59	80.22	20.81	4.53	71.17	69.80	45.78	75.35
4 weeks									
Control	67.40	31.41	81.34	11.18	4.37	21.66	53.66	31.74	78.14
Conditioned	65.73	48.07	98.91	15.77	3.18	28.54	65.08	47.73	94.84
8 weeks									
Control	64.00	37.87	84.23	7.34	3.69	10.30	43.60	16.86	79.21
Conditioned	70.21	37.73	95.96	7.84	1.38	17.34	53.92	35.16	77.62

Median values and upper and lower bounds.
mBIC, mineralized bone–implant contact; OIC, osteoid–implant contact; BV, bone volume.

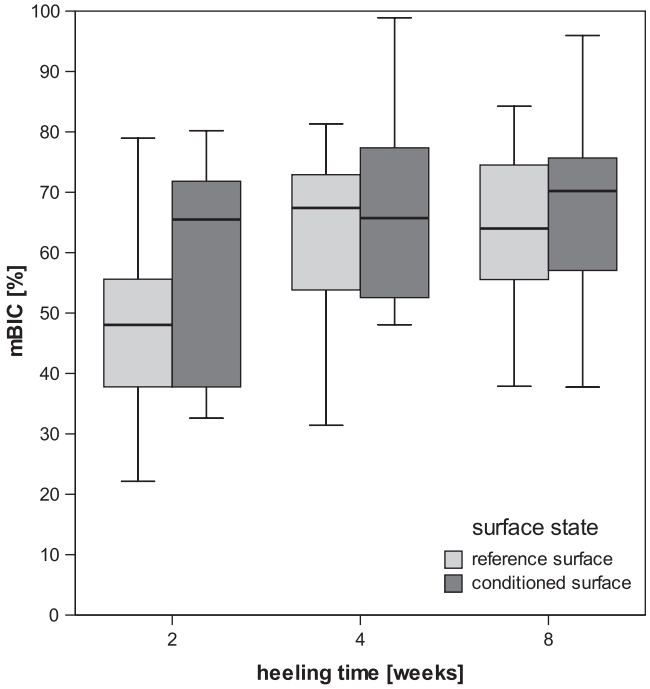

Fig. 9. Mineralized bone–implant contact (mBIC)after 2, 4 and 8 weeks for surface-conditioned and control implants.

OIC levels are rarely determined (Vandamme et al. 2007), a fortiori this parameter enables a prediction of future bone formation. In this study, an equal level of OIC was found after 2 weeks. This was accompanied by differences in BIC, suggesting that a possible osteogenic effect of the surfaces might have taken place earlier. However, a slower decline in OIC after 4 weeks was recorded for surface-conditioned implants. Osteoid production has been described to be closely related to the glycoproteins osteocalcin and osteopontin. It could be supposed that a slightly longer lasting potential for cell differentiation into osteoblasts is present around surface-conditioned implants (Protivinsky et al. 2007). Schwarz et al. (2008) describe increased amounts of osteocalcin around hydrophilic surfaces, supporting a possible osteoblastic differentiation. Nevertheless, this animal study is not designed to confirm the in vitro observations mentioned.

Fluorescence microscopic evaluation provided evidence of bone formation along the implant surface before the fourth week. Both implant surfaces showed the earlier applied alizarin labels closer to the implant surface compared with later applied calceine label. At the same time, there was appositional bone growth within the host bone and towards the implant surface. Overall, no general differences in the dynamics of bone formation between the two surfaces with respect to fluorochrome labels could be found. Because of the tendency of fluorochrome labels to form calcium-bindings, a possible influence on bone growth and mineralization cannot be excluded (Rahn 1976). The absence of tetracycline labels prevented the dynamic evaluation of the 2-week period. The missing label might be due to the dosage and application.

Analysing bone density, BV levels of 70% for surface-conditioned implants and 61% for controls after 2 weeks were determined. Schwarz et al. (2007) reported on BV values, detecting over 40% BV for hydrophilic implants, compared with approximately 35% BV for controls after 2 weeks in an earlier mentioned study (exact numbers not published). Buser et al. (2004) qualitatively describe BV to increase from 2 to 4 and 8 weeks. Such an increase cannot be confirmed by our results, which could be because of processes of remodelling.

There is some evidence that increased bone formation can be achieved with surface-conditioned implants at early periods of healing. However, the question arises as to whether a modification of chemical parameters like hydrophilia further causes changes of other surface parameters that influence bone formation.

In the present study, the surface-conditioned implants were dipped in a conditioning sodium hydroxide solution before implantation. The physicochemical characterization of microrough titanium substrates has shown that a sodium hydroxide solution with a pH value of around 12 is sufficiently basic to alter the physicochemical properties of the substrates to render them hydrophilic. This results in a water contact angle below the detection limit. A comparable effect could not be measured for surfaces dipped into 0.9% saline solution before implantation. Adverse effects of

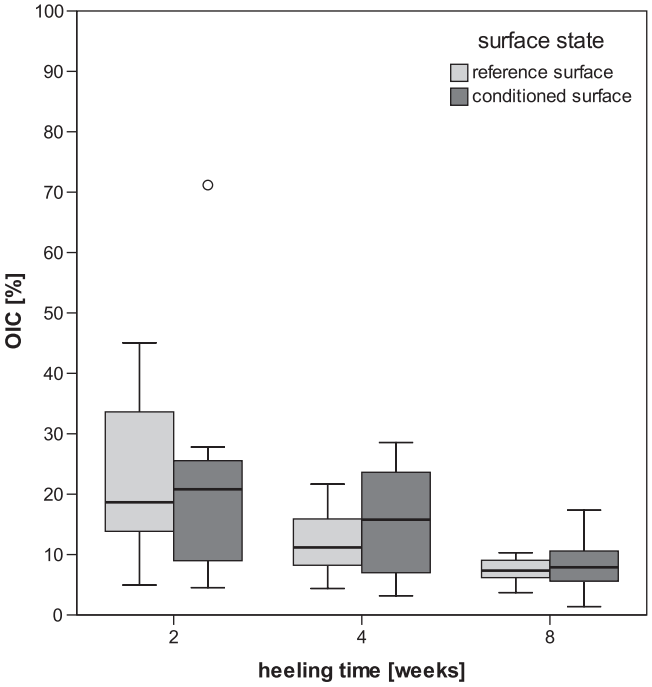

Fig. 10. Osteoid–implant contact (OIC) (osteoid implant contact) after 2, 4 and 8 weeks for surface-conditioned and control implants.

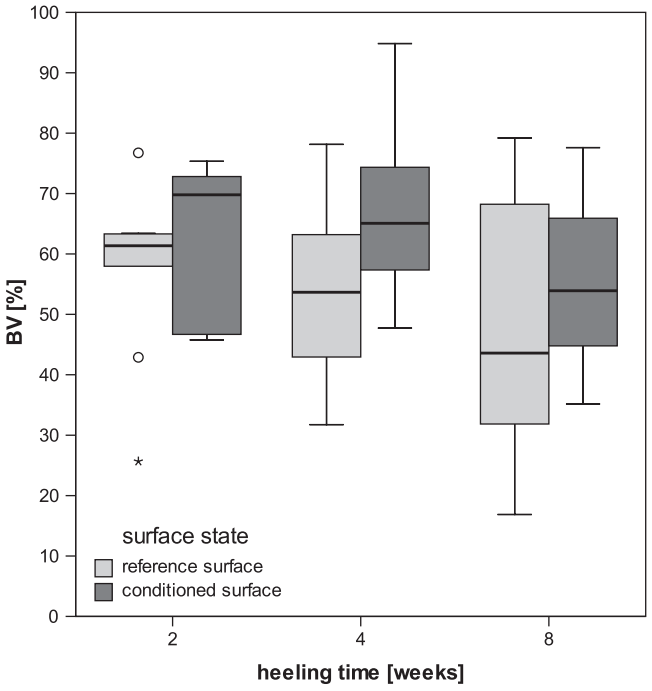

Fig. 11. Bone volume (BV) after 2, 4 and 8 weeks for surface-conditioned and control implants.

residual sodium hydroxide solution after conditioning could be excluded by both, pH measurements directly on the surface after conditioning and haemocompatiblity tests of conditioned surfaces. The former have shown that the residual basic nature of the conditioned surface is rapidly diluted and neutralized. The pH values measured on the surface were not higher than 9 (data not shown). The residual amount of basicity is therefore supposed to be readily neutralized by the hydrogencarbonate buffer system of blood in the implantation site. This hypothesis was underlined by the fact that haemocompatibility parameters of blood did not significantly change after incubation with surface-conditioned implants.

Analysis of topography by X-ray photoelectron spectroscopy (XPS) of microrough titanium substrates that were conditioned with sodium hydroxide and subsequently dried has shown that increased values of hydroxide ions could be detected in comparison with untreated surfaces (data not shown).

Biomaterials with different surface compositions trigger different biologic responses (Sul 2003). The binding of proteins depends on the physicochemical nature of a surface (MacDonald et al. 1998). It is known that in vivo reactions to an implant are mediated by the quantity, homogeneity and functionality of deposited protein films on the implant surfaces (Zhao et al. 2007). While the protein-binding properties of the substrate might influence mainly cell-physiological reactions, the induction of bone bonding could be influenced by the conditioning. It is assumed, that a higher surface free energy initiates such an effect (Rupp et al. 2006). Zhu et al. (2004) describe the importance of surface chemistry in influencing cell attachment, spreading and proliferation. Analysing absorption, fibronectin is described to continuously increase on surfaces created by treatment in a high-temperature and high-concentration sodium hydroxide solution (Protivinsky et al. 2007). Cell attachment and spreading is described to improve remarkably, thus suggesting a substantial influence of wettability. Further, the expression of osteocalcin and osteopontin was increased. However, Protivinsky and colleagues detected no difference in proliferation.

Various in vitro and in vivo studies describe the influence of increased hydrophilia due to surface conditioning on bone-forming processes. Interpreting the data, it is certainly difficult to limit this observation to the singular modification of this parameter. It seems to be undisputed that surface topography has a major influence on bone formation. However, supplementary surface conditioning seems to represent a small but important approach to further influence osseointegration.

In clinical practice, faster bone formation could lead to shorter healing periods as shown for sandblasted acid-etched implants (Weber et al. 2000).

Favourable results in success and survival rates (Roccuzzo et al. 2001) could lead to early-loading protocols without risking implant failure.

Although the data of the present study did not reach statistical significance, a trend towards surface-conditioned implants after short-term healing in non-compromised sites could be observed. This can represent an approach towards shorter healing periods.

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Clinical Relevance

Scientific rationale for the study: This study was designed to analyse possible differences in the dynamics of bone formation around surface-conditioned implants. Increased, earlier bone formation could lead to shorter healing intervals and consecutive earlier implant loading.

Principal findings: Applying an animal model, sandblasted, thermally acid-etched and surface-conditioned implants showed enhanced bone formation, compared with non-surface-conditioned implants. However, despite a trend, there was no significant difference in bone formation. Thus, an effect of this surface treatment is not statistically significantly proven in the present model.

Practical implications: Surface conditioning might further stimulate early osseointegration. This could lead to shorter healing intervals.