

# Effect of intermittent loading and surface roughness on peri-implant bone formation in a bone chamber model

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## Abstract

Both implant surface characteristics and mechanical loading are known to affect implant osseointegration. Their interaction and the underlying mechanisms by which they affect peri-implant healing processes are still unknown. The aim of this study is therefore to investigate the influence of a turned *versus* a rough (Plus<sup>®</sup>, Dentsply Friadent) implant surface on peri-implant bone formation in case of unloaded or loaded implant healing.

**Material and Methods:** Bone formation was evaluated around screw-shaped implants under four experimental conditions using a repeated sampling bone chamber methodology: (1) unloaded turned implant (CU), (2) unloaded implant with a rough surface (TU), (3) loaded turned implant (CL), and (4) loaded implant with a rough surface (TL). Peri-implant tissue samples were paraffin embedded after implant removal and examined histologically and histomorphometrically. A mixed model was used for statistical analysis.

**Results:** The surface of bone tissue relative to the total tissue area (bone area fraction) was not affected by the experimental conditions. The areas of bone trabeculae relative to the bone area (bone fraction) were significantly higher for TL compared with CU and TU. The bone fraction in the vicinity (100  $\mu$ m zone) of the implant (BFZ) was significantly the highest around the loaded roughened implants (TL).

**Conclusion:** Implant loading did not affect bone formation in the absence of surface roughness, and implant surface roughness had no effect in the absence of loading. However, a bone-stimulating effect in the implant's vicinity was assigned to the rough surface when the implant was loaded.

Key words: animal; bone chamber; implant surface roughness; mechanical loading; osseointegration

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Bone surrounding oral implants is sensitive to mechanical loading (Frost 1987, Turner 1998). In line with what

is generally accepted in bone biology, implant loading can stimulate bone formation around the implant to a certain extent, whereas overload leads to peri-implant bone resorption. The same holds true for immediate implant loading where the mechanical loading is superposed on the bone-healing processes. Despite the promising results that are reported (Chiapasco et al. 2001, Romeo et al. 2002, Cannizaro & Leone 2003, Östmann et al. 2005),

immediate implant loading is more critical compared with the conventional delayed implant loading (Ericsson et al. 2000, Engstrand et al. 2003, Wolfinger et al. 2003). This is because bone healing can be threatened by excessive micro-motion between the interfacial tissues and the implant and/or inappropriate loading, leading to high shear forces. A restricted interfacial micro-motion is mentioned to be crucial for the prognosis of immediately loaded

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implants (Szmukler-Moncler et al. 1998). Primary stability plays an important role in limiting this micro-motion and is therefore a key factor in the prognosis of immediately loaded implants (Gapski et al. 2003). This primary stability is mainly a mechanical stability because it is realized by the mechanical support of the surrounding tissues. After tissue healing, the implant stability is substantiated by a biological integration of the implant in its surrounding tissues. Resonance frequency analyses show that there is an initial decrease in implant stability soon after implant installation (Nedir et al. 2004). This dip in implant stability can be explained by peri-implant bone remodelling, also implying bone resorption and therefore loss of implant-supporting tissues. It takes a while before the newly formed bone matures and exhibits mechanical properties similar to the pre-existing bone. As soon as the peri-implant bone has healed, remodelled, and matured, the implant is considered to be biologically stable. Because implant stability is decisive in the prognosis of immediately loaded implants, we need to pursue an optimal primary mechanical stability and an as fast as possible biological integration.

Implant surface roughness leads after healing to an increased mechanical stability through surface enlargement and mechanical interlocking with the surrounding bone. In addition, some studies indicate that surface roughness as such also promotes a biological effect. Implant surface topography can indeed influence the cell's shape, orientation, motion, and even function (Qu et al. 1996, Brunette & Chehroudi 1999). This was demonstrated in several studies (Wennerberg et al. 1995, Novaes et al. 2002, Abrahamsson et al. 2004, Botticelli et al. 2005). To set an example, the study by Abrahamsson et al. (2004) evaluated the rate and degree of osseointegration at turned (T) versus rough (sand blasted and acid etched) implant surfaces during the early phases of healing. The healing characteristics were similar, but the degree as well as the rate of osseointegration was superior for the rough compared with the turned implants.

A favourable biomechanical coupling or load transfer of the occlusal forces towards the bone improves the implant's prognosis (Wiskott & Belser 1999). The bone-stimulating potential of an

adequate biomechanical coupling, e.g. provided by surface roughness, is demonstrated by Piatelli et al. (1998). Their study was conducted on 12 macaca fascicularis in which they installed 48 titanium screw-shaped, plasma-sprayed implants. Twenty-four were placed in the posterior maxilla and the other 24 in the posterior mandible. At each site, half of the implants were early loaded (after 3 days) whereas the other half remained unloaded. The histological data revealed a higher bone-to-implant contact (BIC) for the loaded (maxilla:  $67.3 \pm 7.6\%$ , mandibula:  $73.2 \pm 5.9\%$ ) compared with the unloaded (maxilla:  $54.5 \pm 3.3\%$ , mandibula:  $55.8 \pm 6.5\%$ ) implants after 9 months of implant healing. This study could, however, not identify the sole effect of immediate implant loading or surface roughness, though, as neither unloaded nor loaded smooth implants were included as controls. The latter was aimed at in a study of Rocci et al. (2003), who investigated the effect of surface roughness in case of immediate implant loading in a randomized clinical trial. They found a better survival rate for the rough compared with the machined surface, although the difference was not statistically significant.

This study aimed to explore the effect of implant surface roughness on the pace of peri-implant bone formation and to evaluate whether well-controlled loading affects this process. It was hypothesized that implant surface roughness accelerates the peri-implant bone formation.

## Material and Methods

### Bone chamber methodology (Duyck et al. 2004)

The bone chamber model (Fig. 1) primarily consists of two hollow cylinders, which fit exactly into each other. In a first stage, the outer cylinder – called the outer bone chamber (a) – is installed in the proximo-medial tibia of five New Zealand White rabbits under aseptic conditions and is allowed to osseointegrate. The bone chamber has an outer diameter of 1 cm and on average 9 mm – of a total height of 12 mm – is inserted into the bone. During this healing period, the outer bone chamber is filled with a solid teflon cylinder (h) to prevent tissue growth via the three perforations (c). After 3 months of healing, this teflon cylinder is removed and replaced

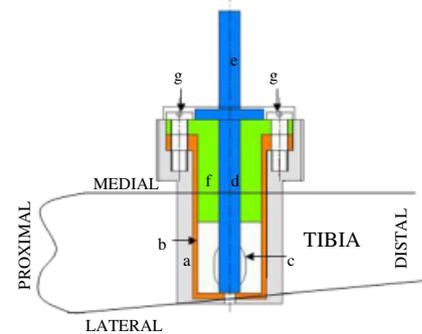


Fig. 1. Schematic drawing of the bone chamber model. (a) outer bone chamber, (b) inner bone chamber, (c) perforation, (d/e) experimental implant, (f) Teflon bearing, (g) set screw and (h) Teflon cylinder.

by an inner cylinder, called the inner bone chamber (b). This inner bone chamber is in close contact with the outer bone chamber and has perforations (c) that match with the latter. This allows tissue growth into the central cavity of the bone chamber. The implant (d), located in the centre of the bone chamber, is displaced in a well-controlled manner by means of an external loading device (actuator), which is attached to the implant at point 'e'. During implant loading, the implant slides in a teflon bearing (f). Several screws (g) connect all components. After the experiment, the implant and its surrounding tissues are harvested by removing the inner from the outer bone chamber. A next experiment can start afterwards with a new inner bone chamber placed in the outer one. This allows for several experiments (harvests) within the same animal.

The loading device or actuator that mechanically loaded the implants consists of a piezo translator (pre-loaded closed-loop LVPZT translator, P-841.60; ALT, Eindhoven, the Netherlands), which can induce a displacement of up to 90  $\mu\text{m}$ , and a load cell (XFTC 100-M5M-1000N; GS Sensors SARL, Les Clayes sous Bois, France) with a capacity of 1000 N in tension and 100 N in compression. The displacement is controlled by a strain gauge onto the piezo stack itself. A closed-loop control assures the required displacement. The displacement can be applied with a frequency of up to 50 Hz. The actuator is controlled by software written in Test Point (Norton, MA, USA). The load parameters that were defined for this study were displacement of the implant (30  $\mu\text{m}$ ), load frequency (1 Hz), and number of load cycles (400).

Both input (defined load parameters) and output (applied forces on and displacement of the implant) are registered and visualized on the computer screen during load application, and saved to a file. For the researcher's convenience, the animal is immobilized during load application. Additional movement of the animal did not influence the implant loading once the actuator was connected to the implant.

#### Animal information

Before the outer bone chamber installation, the animals were pre-anaesthetized with Ketamine (Ketamine 1000 CEVA<sup>®</sup>, 0.12 ml/kg body weight; Ceva Sante Animale, Brussels, Belgium) and Xylazine (Vexylan<sup>®</sup>, 0.12 ml/kg body weight; Sanofi, Machelen, Belgium). During the surgery, they were given Propofol (Diprivan<sup>®</sup> 1% – 8 ml/h; Astra Zenica, Brussels, Belgium) as an intravenous anaesthetic. Post-operatively, the animals were given Buprenorfin as analgesics (Temgesic<sup>®</sup>, Reckit & Coleman, Richmond, VA, USA; 0.05 mg/kg body weight I.M.), and antibiotics (300,000 IU/Injection I.M.-ad perf./ Benzylpenicillium natricum 600 mg/Continental Pharma, Belgium) were administered pre-operatively and 4 consecutive days post-operatively. For harvesting of the inner bone chamber and replacement by a new one, the animals were anaesthetized with Ketamine and Xylazine. The experiments were approved by the local ethical committee for laboratory animal science and were performed according to the Belgian animal welfare regulations and guidelines.

#### Study design

Two sets of six mature female New Zealand white rabbits were used for this study. A first set of six animals received a bone chamber. One of these animals died during the outer bone chamber healing due to an intestinal infection. Owing to the re-usability of the outer bone chamber, the first three experiments were performed within the same set of five animals. After each experiment, the inner bone chamber with its content was harvested and a new one was inserted to allow a next experiment.

Because the interpretation of the results of these first three experiments required an additional experiment, a fourth experiment was conducted in

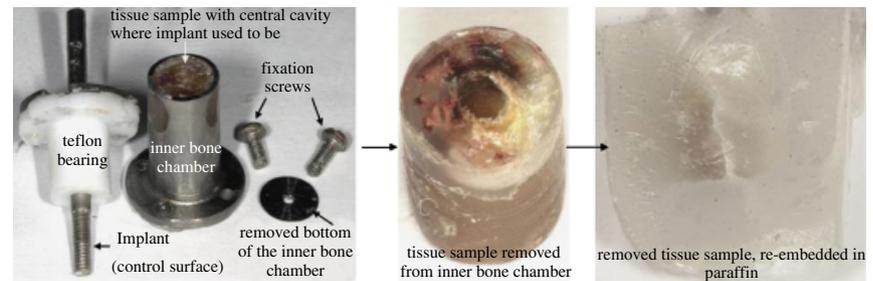


Fig. 2. The inner bone chamber content was freed from the metal parts and re-embedded in paraffin before histological sectioning.

bone chambers placed in the second set of six animals.

The four experimental conditions were as follows:

- Experiment 1: rough (test) implant, no mechanical loading.
- Experiment 2: turned (control) implant, no mechanical loading.
- Experiment 3: rough (test) implant, mechanical loading.
- Experiment 4: turned (control) implant, mechanical loading.

Each of the four experiments lasted for 6 weeks.

All implants were screw-shaped (M2) and custom made from c.p. titanium. The test implants were SLA (Plus<sup>®</sup> surface; Dentsply Friadent, Mannheim, Germany), resulting in an  $R_a$  value of  $2.75 \mu\text{m}$ . The control implants were decontaminated by immersing them in HF (4%) for 30 s, after which they were neutralized in 20%  $\text{HNO}_3$ , rinsed with demineralized water, cleaned with methanol (100%), and sterilized. Scanning electron microscopy (SEM) analyses failed to detect the remaining  $\text{F}^-$  ions on the implant surfaces. The latter implants had an  $R_a$  value of  $0.45 \mu\text{m}$ .

Mechanical loading of the implants was initiated the same day as the implant installation and was performed three times a week for a period of 6 weeks. The loading sessions were organized as such that the time intervals between the loading sessions were kept constant.

#### Histology and histomorphometry

Immediately after sacrificing the animals, all 21-tissue samples, retrieved from the inner bone chambers, were fixed in 2% paraformaldehyde. These tissue blocks were decalcified in 0.5 M EDTA (pH 7.4)/PBS at 4°C before dehydration and embedded in paraffin.

The implants were gently removed from the paraffin-embedded tissue blocks and the tissue plug was removed from the inner bone chamber after removing the inner bone chamber bottom (Fig. 2). The effect of this implant removal was evaluated in a previous study (Slaets et al. 2006) by SEM. Only very few small sponge-like structures without cells were observed, probably consisting of fibrous tissue or cell remnants. The remaining surface of the implants was comparable with the surface of a sterile implant. The samples were re-embedded immediately after implant removal to refill the central cavity and cut to 4- $\mu\text{m}$ -thick sections perpendicular to the implant axis (Fig. 2).

Three sets of 10 sections each were taken per sample. The first set was taken in the second 0.5 mm starting from the bottom of the inner bone chamber. This is the most apical region around the central implant. The second and third sets were taken 1.5 and 3 mm higher than the first set. With the sections taken in a window of 3 mm at the level of the bone chamber perforations (4 mm in height), they well represent the tissues surrounding the experimental implant.

Several stainings were performed per set:

- Haematoxylin and eosin (H & E) staining was performed for general morphological analysis (Fig. 3).
- Osteoclasts were visualized by tartrate-resistant acid phosphatase (TRAP) activity and counterstained with Light Green SF Yellowish (Fig. 4).
- CD31 staining was performed for the visualization of the vascularization (endothelial cells) (Fig. 5).
- Monoclonal antibody staining for Runx2/Cbfa1 gene expression was performed for the detection of early osteoblast differentiation (Bronckers

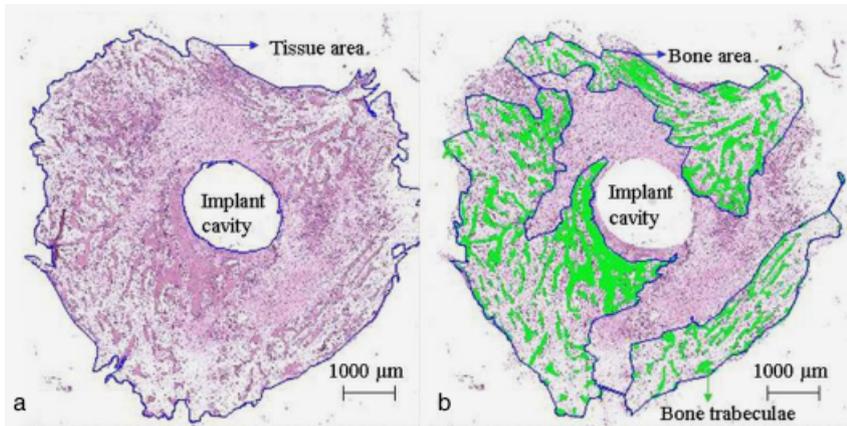


Fig. 3. Haematoxylin & eosin-stained section cut perpendicular to the implant axis, showing the central cavity where the implant used to be. The tissue area represents all available tissue in the bone chamber, outlined in blue on the left-hand picture (a). The bone area fraction (BAF) is the area occupied by the bone tissue relative to the tissue area, outlined in blue on the right-hand picture (b). The bone fraction (BF) is the area occupied by all bone trabeculae (indicated in green) relative to the area occupied by the bone tissue.

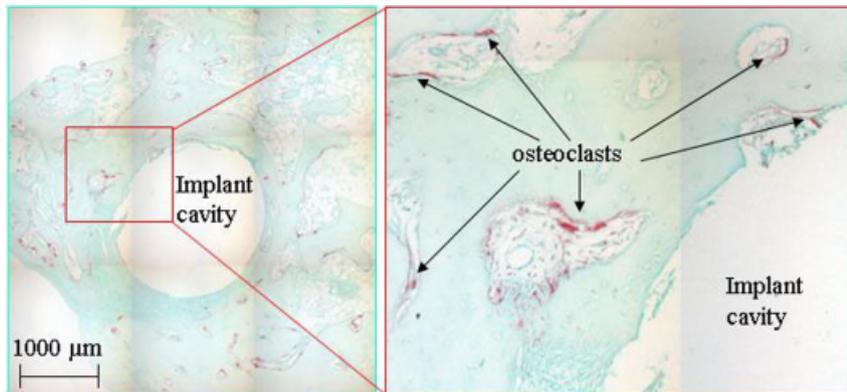


Fig. 4. TRAP-stained section showing bone-resorbing cells or osteoclasts (red). TRAP, tartrate-resistant acid phosphatase.

et al. 2003). The immunopositive cells stain dark brown, whereas the immunonegative cells stain blue. (Fig. 6).

- Per set of 10 sections, each of the four stainings was applied on two sections. The remaining two sections were spare ones. The selection between both sections of one particular staining was based on the quality of the sections.

Histological analyses were performed to generally describe the tissues surrounding the implants. The histomorphometrical analyses were performed by means of a light microscope (Leitz Laborlux S, Wetzlar, Germany) connected to a PC, equipped with a video (Pro-Series high-performance CCD camera, Media Cybernetics, L.P., Bethesda, MD, USA) and image analy-

sis system (Image Pro Plus<sup>®</sup> 3.0.01.00, Media Cybernetics, L.P.). The image processing was automated. All measurements were manually checked and redressed if necessary.

The following parameters were measured on the H & E-stained histological sections:

- Bone area fraction (BAF%) = surface occupied by bone (= bone trabeculae, together with the interstitial tissues) relative to the tissue area (Fig. 3).
- Bone fraction (BF%): summation of all areas occupied by individual bone trabeculae relative to the total bone area (bone trabeculae + interstitial tissues) (Fig. 3).
- Bone Fraction in the 100 µm zone around the implant cavity (BFZ%): summation of all areas occupied by

individual bone trabeculae relative to the tissue surface in the 100 µm zone around the implant cavity.

The summation of all areas occupied by osteoclasts was measured on the TRAP-stained sections and its share was calculated relative to the areas occupied by bone trabeculae (OCI%) (Fig. 4).

The degree of vascularization was measured on the CD31-stained histological sections (Fig. 5):

- Vessel fraction in bone (VFB%): summation of all areas occupied by blood vessels relative to the bone area in a randomly chosen reference area.
- Vessel fraction in bone marrow (VFBM%): summation of all areas occupied by blood vessels relative to the bone marrow area in a randomly chosen reference area.

The origin of the sections was blinded during histological and histomorphometrical analyses.

**Statistics**

The data were statistically analysed by means of both the linear mixed model and the logistic mixed model (Verbeke & Molenberghs 2000, 2005) using SAS (SAS/STAT software, SAS Institute Inc., NC, USA). The procedure proc mixed was used to fit the model. Because the rabbit-specific effect is random, it was modelled accordingly. The other variables such as experimental condition, number of histological section, or harvest number are fixed effects. Overall, the level of significance was set with  $\alpha = 0.05$ . However, for some comparisons, this was set to a lower level because a multiple-comparison procedure was used.

**Results**

The histological sections revealed a healthy tissue inside the bone chamber, consisting mainly of bone tissue (60–80%). The bone was generally formed by bone apposition from the host bone growing into the chamber via the perforations into the bone chamber. Osteoblasts lining the bone trabeculae were indicative of processes of active bone apposition. The tissues were highly vascularized (Fig. 5) and also the bone

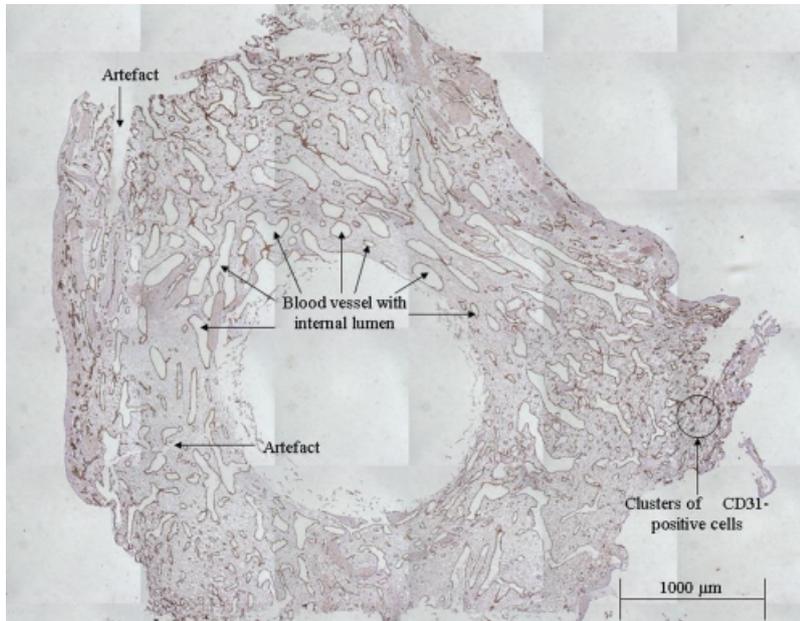


Fig. 5. CD31-stained histological section visualizing the tissue vascularization. The endothelial cells of the blood vessels are stained dark brown.

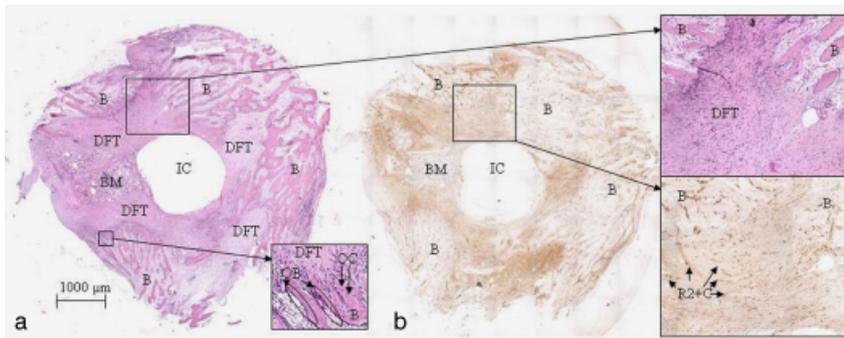


Fig. 6. Consecutive paraffin sections, stained with hematoxylin & eosin (a) and stained for Runx2 gene expression (b). (a) Shows bone tissue (B), bone marrow (BM), and dense fibrous-like tissue (DFT). This cell-rich fibrous-like tissue contains numerous cells that express the Runx2 gene (R2+C), which is a master gene for osteoblast differentiation. Osteoblasts (OB) lining the bone trabeculae (containing the osteocytes – OC) are indicative of processes of active bone apposition.

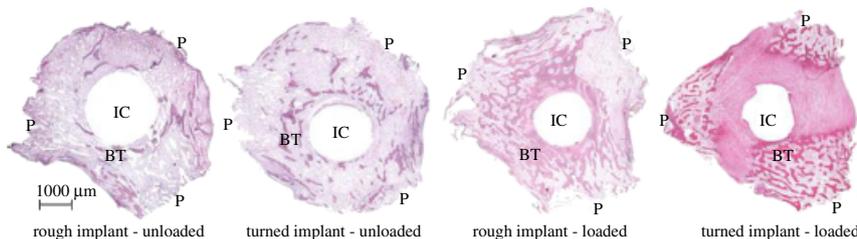


Fig. 7. Representative sections of the four experimental conditions. The left three sections are retrieved from the same animal. IC, implant cavity; BT, bone tissue; P, position of a perforation.

marrow and a fibrous-like tissue were observed (Fig. 6). This fibrous-like tissue was continuous with the bone and had a dense appearance. This type of tissue contained numerous cells expres-

sing the Runx2/Cbfa1 gene, which is indicative of early osteoblast differentiation (Fig. 6).

In most of the samples, a haematopoietic bone marrow was observed con-

taining megakaryocytes, lymphocytes, polymorphonuclear cells, plasmacells, and macrophages. No signs of active inflammation were noticed though. Besides blood vessels with a central lumina, and also small clusters of CD31 immunopositive cells were observed (Fig. 5). These were not included in the measurements for the vascularization.

Figure 7 illustrates representative sections for the four experimental conditions. The mean values ( $\pm$  standard deviations) of the parameters measured histomorphometrically are summarized in Table 1. The proportion of bone relative to all tissues in the bone chamber (BAF) did not differ statistically between the experimental conditions. The bone fraction (BF), representing the bone density, was higher around the loaded compared with the unloaded implants. This difference was only significant between the loaded test implants and the unloaded implants. The fraction of bone in the 100  $\mu$ m zone around the loaded test implants, however, was significantly higher than the other experimental conditions.

The share of osteoclasts in the bone tissue was similar in all the experimental conditions.

Significantly more blood vessels were observed in the bone marrow compared with the bone. Within each tissue, however, the vascularization was not affected by the experimental condition.

## Discussion

This study was designed to evaluate the effect of surface roughness in addition to implant loading on the peri-implant bone formation.

The peri-implant tissue response was not affected by the surface roughening in the absence of loading. Mechanical loading, however, stimulated bone formation and therefore led to higher bone fractions, particularly in the implant's vicinity. Despite a clear tendency towards higher bone formation around all loaded implants, the increase in bone fractions was only significant for the loaded roughened implants. The bone fraction in the 100  $\mu$ m zone around the loaded implants was significantly higher for the roughened compared with the smooth implants, which supports the hypothesis that implant surface roughness can accelerate the peri-implant bone formation.

Table 1. Mean values ( $\pm$  standard deviations) of the bone area fraction (BAF), the bone fraction (BF), the bone fraction in the 100  $\mu$ m zone around the implant cavity (BFZ), the proportion of osteoclasts (Ocl), the vascularization in the bone (VFB) and the vascularization in the bone marrow (VFBM)

Experiment	BAF (%)	BF (%)	BFZ (%)	Ocl (%)	VFB (%)	VFBM (%)
Test unloaded	78.7 ( $\pm$ 33)	20.3 ( $\pm$ 7.3)*	14.8 ( $\pm$ 14.1)*	0.52 ( $\pm$ 0.52)	24.1 ( $\pm$ 10.1)	33.9 ( $\pm$ 8.8)
Control unloaded	66.2 ( $\pm$ 34.5)	20.6 ( $\pm$ 15.9) $\circ$	17.7 ( $\pm$ 18.1) $\circ$	0.67 ( $\pm$ 0.69)	17.4 ( $\pm$ 11.5)	33.7 ( $\pm$ 8.3)
Test loaded	65.2 ( $\pm$ 16)	40.9 ( $\pm$ 8)* $\circ$	70.7 ( $\pm$ 28.6)* $\circ$ $\blacklozenge$	0.33 ( $\pm$ 0.28)	13.1 ( $\pm$ 12.9)	24.8 ( $\pm$ 2.3)
Control loaded	61 ( $\pm$ 22.7)	31.6 ( $\pm$ 6.6)	24 ( $\pm$ 24.97) $\blacklozenge$	0.29 ( $\pm$ 0.1)	11.9 ( $\pm$ 8.4)	12.8 ( $\pm$ 7.5)

For each parameter (within each column), the values that differ significantly are marked with equal symbols (\*,  $\circ$ , or  $\blacklozenge$ ).

The hypothesis is, however, in part rejected as implant surface roughening had no effect in the absence of loading. A similar bone chamber study by Vandamme et al. (2007c) also revealed no difference between smooth and rough implants in an unloaded situation when considering the percentage of BIC. The incidence of BIC, though, was significantly higher for the rough implants. Also, other studies (Wennerberg et al. 1995, Novaes et al. 2002, Abrahamsson et al. 2004, Botticelli et al. 2005) indicate that implant surface roughness can have a bone-stimulating effect. Although no direct occlusal load was applied on the implants in the latter studies, the implants were not isolated from indirect loads (bending of the whole bone during physiological function, muscle tension, etc.) as was the case in this study. The complete lack of mechanical stimulation of the tissues within the isolated bone chamber environment might explain the lower bone formation under the unloaded test conditions in this study.

It is generally accepted that mechanical loading stimulates bone formation if it does not exceed certain values (Frost 1987, Turner 1998). This also holds true for bone surrounding implants. On the other hand, it is known that micro-motion at the bone-implant interface has a detrimental effect on peri-implant bone (Szmukler-Moncler et al. 1998). It seems that the combination of an appropriate implant macro-design (screw shape), micro-design (surface roughening), and mechanical loading leads to stimulation of peri-implant bone formation. The screw-shaped implant design and the surface roughness both provide the implant with a solid interlocking with its surrounding tissues. This interlocking prevents interfacial micro-motion and promotes an efficient force transfer in case of implant loading. This phenomenon points to the biomechanical coupling as mentioned by Wiskott & Belser (1999). They underline the detri-

mental effects of lack of function e.g. around smooth implant collars due to stress shielding.

A lack of biomechanical coupling was illustrated by a similar bone chamber study with turned cylindrical implants (Duyck et al. 2006). No bone-stimulating effect was seen as a result of implant loading after 6 weeks. In the latter, the implant's macro- and micro-design did not provide a good interlocking with the surrounding tissues, leading to an inefficient force transfer and interfacial micro-motion. Continuing the same experiment for another 6 weeks, on the other hand, did reveal a bone-stimulating effect from the same implant configuration (Vandamme et al. 2007b). It seems that implant loading can only have a stimulating effect in case of a good biomechanical coupling. This coupling can be realized by interlocking through macro- and micro-design as well as by the BIC itself. The latter is thought to be the case in the 12-week experiment (Vandamme et al. 2007b). As soon as minor BIC has been established, bone-implant micro-motion decreases at the interface and implant loading is transferred towards the surrounding tissues, thereby stimulating bone formation.

Clinical (Schincaglia et al. 2007) and experimental (Piatelli et al. 1998, De Smet et al. 2005, Vandamme et al. 2007a-c) data support the idea that peri-implant bone benefits from an efficient load transfer from the load-bearing implant towards the tissues, which is confirmed in this study. Also, a recent study by Leucht et al. (2007) underlines the bone-stimulating effect of mechanical implant loading. In that study, 0.5-mm-wide polymer pin-shaped implants were placed in 0.8-mm-wide bone cavities in mouse tibiae. The implant's pin shape allowed a good biomechanical coupling, resulting in an enhanced peri-implant bone formation in response to the mechanical implant loading. The distance between the

implant surface and bone matrix deposition varied on average between 10 and 90  $\mu$ m and depended on the local effective strains. The areas of high effective strains revealed the greatest distance.

In all 21 samples from this study, the initial blood clot evolved into highly vascularized tissue consisting of bone tissue, bone marrow, fibrous tissue, and tissue containing cells that were likely to be differentiating into the osteoblastic cell lineage. Whereas bone, bone marrow, vessels, etc. can be identified with reasonable certainty, the bone chambers also contained tissues that were harder to identify. All samples exhibited a dense fibrous-like tissue that at certain places seemed to be continuous with the bone tissue. This tissue presented along with bone trabeculae lined with active osteoblasts, suggesting that the conditions for bone formation were favourable. To check the osteogenic potential of this tissue, a staining for the expression of Runx2/Cbfa1 was performed. Runx2/Cbfa1 is a transcription factor, essential for differentiation of osteoblasts from undifferentiated progenitor cells (Karsenty et al. 1999, Ducy et al. 2001). The osteoblasts lining the bone trabeculae-stained immunopositive as well as most of the embedded osteocytes. Also, in the dense fibrous-like tissue, many cells were immunopositive. Because similar observations were made during the intra-membranous jaw bone and calvaria formation (Bronckers et al. 2003), this dense fibrous-like tissue might be indicative of de novo bone formation. This is an indication but no proof for intramembranous bone formation, because a recent publication reports on the expression of Runx2/Cbfa1 in a multipotential mesenchymal cell population giving rise to both osseous and non-osseous cell lineages (Bronckers et al. 2005). Although the amount of connective tissue containing the Runx2/Cbfa1 immunopositive cells was quantified, it was not reported or

used for comparison of the different experimental conditions because of its ambiguous interpretation.

The interpretation of bone tissue, on the other hand, was clear. Because a tight connection between implant and surrounding bone is a prerequisite of osseointegration, the amount of bone in the implant vicinity was taken as a measure of osseointegration. The percentage of BIC, which is usually taken as a measure for osseointegration, could not be quantified due to the implant removal. Whereas tissue loss at the interface due to implant removal can affect the percentage of bone-to-implant cavity contact, it will have a minor effect on the proportion of the tissues in a broader zone around the implant. The bone fraction in the 100  $\mu\text{m}$  zone around the implant was therefore used as a measure of peri-implant bone formation.

The surface occupied by osteoclasts relative to the bone tissue surface, which is indicative for the bone resorption rate, was about double under the unloaded conditions compared with the loaded conditions. Also, the share of blood vessels was consistently higher under the unloaded conditions. This indicates a higher bone-remodelling rate for the unloaded test conditions. This seems to be contradictory but might be explained as the possibly faster maturation of the tissues surrounding the loaded implants. Despite the large differences in the percentage of osteoclasts and blood vessels between the experimental conditions, these were, however, not statistically significant.

The vascularization in all samples was abundant. Besides the blood vessels, which could be clearly identified as lumina surrounded by CD31 immunopositive endothelial cells, there were also small clusters of immunopositive cells without the central lumina. These could be tiny or sprouting microvessels. As CD31 can also be expressed diffusely on the surfaces of megakaryocytes, myeloid cells, natural killer cells, and some subsets of T-cells and B-cell precursors (Muller 1997), these were, however, not taken into account as part of the vascularization.

The bone chamber methodology was used to control the mechanical conditions in the implant surroundings. The bone chamber fully protects the tissues in its lumen from external influencing factors, which allows investigation of the effect of specific parameters on the tissue response around the implanted

material. In addition, the possibility of repeated sampling diminishes the effect of site, subject, and species dependency of the tissue response because several experiments can be conducted at the same site in the same animal.

The endeavour to control the mechanical peri-implant conditions, however, makes the bone chamber set-up differ from the clinical situation. Indeed, the bone chamber conditions are definitely different in case the implants are installed in healed bone sites, but mimic the situation where implants are installed in fresh extraction sites, leaving a gap between the implant and the host bone as there is an initial gap of 2.5 mm in the bone chamber to be bridged between the implant and the bony implant bed. This implies that the implants are initially surrounded by only a blood clot, without any direct host bone contact. This results in more time needed to arrive at implant osseointegration, on the one hand, but it allows a good evaluation of the tissue-healing processes and bone formation on the other. The signs of active bone formation indicate that the processes of cell differentiation and tissue formation did not reach an equilibrium yet. This implies that the results of this study deal more with the process of osseointegration rather than with the final implant osseointegration status. However, the duration of the experiments was kept short on purpose to be able to intercept the influence of the implant loading and surface roughness on the process of cell differentiation and tissue formation. If the experiments had lasted until all experimental conditions would have reached an equilibrium, there was a chance that eventually all four experimental conditions reached the same end situation, thereby blurring the effect of implant loading and surface roughness. As a consequence of the limited healing time, the absolute values of the histomorphometrical parameters should not be compared with those from other studies. They only serve to compare the four experimental conditions of this particular study.

At the time of the start of the study, the number of experiments within the same bone chambers was limited to three for reasons of prudence concerning possible problems of infection and exhaustion. No signs of infection were observed, though, and the results of previous bone chamber studies revealed no exhaustion effect after repeated bone

chamber sampling (Duyck et al. 2006, Vandamme et al. 2007a). In these three experiments, the effect of implant surface roughness was evaluated in the absence of loading and compared with the situation of implant roughening in combination with immediate implant loading. The fact that bone formation was stimulated in case of implant roughening and loading made it impossible to distinguish the sole effect of implant roughening and immediate implant loading. For this reason, an additional experiment (loaded turned implant) was added on a new set of animals. Although the latter animals came from the same nest as the first set of animals, the fact that different animals were used for experiment 4 was taken strictly into account in the statistical analyses.

This study revealed that there is indeed an effect of implant loading and surface roughening on the peri-implant bone formation. More fundamental research, however, is needed to further unravel the mechanisms through which mechanical loading and surface roughness affect peri-implant bone healing. Also, the influence of the very nature of the mechanical loading (load magnitude, frequency, etc.) and the phase of the healing process that it is interacting with are important issues to be addressed.

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### References

- Abrahamsson, I., Berglundh, T., Linder, E., Lang, N. P. & Lindhe, J. (2004) Early bone formation adjacent to rough and turned endosseous implant surfaces. An experimental study in the dog. *Clinical Oral Implants Research* **15**, 381–392.
- Botticelli, D., Berglundh, T., Persson, L. G. & Lindhe, J. (2005) Bone regeneration at implants with turned or rough surfaces in self-contained defects. An experimental study in the dog. *Journal of Clinical Periodontology* **32**, 448–455.
- Bronckers, A., Sasaguri, K. & Engelse, M. A. (2003) Transcription and immunolocalization of Runx2/Cbfa1/Pebp2 $\alpha$ A in developing rodent and human craniofacial tissues: further evidence suggesting osteoclasts phagocytose osteocytes. *Microscopy Research and Technique* **61**, 540–548.

- Bronckers, A. L. J. J., Sasaguri, K., Cavender, A. C., D'Souza, R. N. D. & Engelse, M. A. (2005) Expression of Runx2/Cbfa1/Pebp 2 $\alpha$ A during angiogenesis in postnatal rodent and fetal human orofacial tissues. *Journal of Bone and Mineral Research* **20**, 428–437.
- Brunette, D. M. & Chehroudi, B. (1999) The effects of the surface topography of micro-machined titanium substrata on cell behavior in vitro and in vivo. *Journal of Biomechanical Engineering* **121**, 49–57.
- Cannizaro, G. & Leone, M. (2003) Restoration of partially edentulous patients using dental implants with a microtextured surface: a prospective comparison of delayed and immediate full occlusal loading. *International Journal of Oral and Maxillofacial Implants* **18**, 512–522.
- Chiapasco, M., Abati, S., Romeo, E. & Vogels, G. (2001) Implant-retained mandibular overdentures with Brånemark System MKII implants: a prospective comparative study between delayed and immediate loading. *International Journal of Oral and Maxillofacial Implants* **16**, 537–546.
- De Smet, E., Jaecques, S., Vandamme, K., Vander Sloten, J. & Naert, I. (2005) Positive effect of early loading on implant stability in the bi-cortical guinea-pig model. *Clinical Oral Implants Research* **16**, 402–407.
- Ducy, P., Starbuck, M., Priemel, M., Shen, J., Pinerro, G., Geoffroy, V., Amling, M. & Karsenty, G. (2001) A Cbfa1-dependent genetic pathway controls bone formation beyond embryonic development. *Genes and Development* **13**, 1025–1036.
- Duyck, J., De Cooman, M., Puers, R., Van Oosterwyck, H., Vander Sloten, J. & Naert, I. (2004) A repeated sampling bone chamber methodology for the evaluation of tissue differentiation and bone remodeling around titanium implants under controlled mechanical conditions. *Journal of Biomechanics* **37**, 1819–1822.
- Duyck, J., Vandamme, K., Geris, L., Van Oosterwyck, H., De Cooman, M., Vandersloten, J., Puers, R. & Naert, I. (2006) The influence of micro-motion on the tissue differentiation around immediately loaded cylindrical turned titanium implants. *Archives of Oral Biology* **51**, 1–9.
- Engstrand, P., Grondahl, K., Ohmell, L. O., Nilsson, P., Nannmark, U. & Branemark, P. I. (2003) Prospective follow-up study of 95 patients with edentulous mandibles treated according to the Brånemark Novum concept. *Clinical Implant Dentistry and Related Research* **5**, 3–10.
- Ericsson, I., Nilson, H., Lindh, T., Nilner, K. & Randow, K. (2000) Immediate functional loading of Brånemark single tooth implants. An 18 months' clinical pilot follow-up study. *Clinical Oral Implants Research* **11**, 26–33.
- Frost, H. M. (1987) Bone "mass" and the "mechanostat". A proposal. *Anatomical Record* **219**, 1–9.
- Gapski, R., Wang, H. L., Mascarenhas, P. & Lang, N. (2003) Critical review of immediate implant loading. *Clinical Oral Implants Research* **14**, 515–527.
- Karsenty, G., Ducy, P., Starbuck, M., Priemel, M., Shen, J., Geoffroy, V. & Amling, M. (1999) Cbfa1 as a regulator of osteoblast differentiation and function. *Bone* **25**, 107–108.
- Leucht, P., Kim, J. B., Wazen, R., Currey, J. A., Nanci, A., Brunski, J. B. & Helms, J. A. (2007) Effect of mechanical stimuli on skeletal regeneration around implants. *Bone* **40**, 919–930.
- Muller, W. A. (1997) CD31 workshop panel report. In: Kishimoto, T., Kikutani, H., von dem Borne, A. E. G., Goyert, S. M., son, D. Y. & Miyasaka, M. (eds). *Leucocyte Typing VI. White Cell Differentiation Antigens. Proceedings of the 6th International Workshop and Conference. 1999 Nov 10–14; Kobe*, pp. 362–364. New York: Garland Publishing Inc.
- Nedir, R., Bischof, M., Szmukler-Moncler, S., Bernard, J.-P. & Samson, J. (2004) Predicting osseointegration by means of implant primary stability. A resonance-frequency analysis study with delayed and immediately loaded ITI SLA implants. *Clinical Oral Implant Research* **15**, 520–528.
- Novaes, A. B. Jr, Souza, S. L., de Oliveira, P. T. & Souza, A. M. (2002) Histomorphometric analysis of the bone-implant contact obtained with 4 different implant surface treatments placed side by side in the dog mandible. *International Journal of Oral and Maxillofacial Implants* **17**, 377–383.
- Östmann, P. O., Hellman, M. & Sennerby, L. (2005) Direct implant loading in the edentulous maxilla using a bone density-adapted surgical protocol and primary implant stability criteria for inclusion. *Clinical Implant Dentistry and Related Research* **7** (Suppl. 1), S60–S69.
- Piatelli, A., Corigliano, M., Scarano, A., Costigliola, G. & Paolantonio, M. (1998) Immediate loading of titanium plasma-sprayed implants: an histologic analysis in monkeys. *Journal of Periodontology* **69**, 321–332.
- Qu, J., Chehroudi, B. & Brunette, D. M. (1996) The use of micromachined surfaces to investigate the cell behavioural factors essential to osseointegration. *Oral Diseases* **2**, 102–115.
- Rocci, A., Martignoni, M. & Gottlow, J. (2003) Immediate loading of Brånemark System TiUnite and machined-surface implants in the posterior mandible: a randomized open-ended clinical trial. *Clinical Implant Dentistry and Related Research* **5** (Suppl. 1), 57–63.
- Romeo, E., Chiapasco, M., Lanza, A., Casentini, P., Ghisolfi, M., Iorio, M. & Vogel, G. (2002) Implant-retained mandibular overdentures with ITI Implants. *Clinical Oral Implants Research* **13**, 495–501.
- Schincaglia, G. P., Marzola, R., Scapoli, C. & Scotti, R. (2007) Immediate loading of dental implants supporting fixed partial dentures in the posterior mandible: a randomized controlled split-mouth study – machined versus titanium oxide implant surface. *International Journal of Oral and Maxillofacial Implants* **22**, 35–46.
- Slaets, E., Carmeliet, G., Naert, I. & Duyck, J. (2006) Early cellular responses in cortical bone healing around unloaded titanium implants: an animal study. *Journal of Periodontology* **77**, 1015–1024.
- Szmukler-Moncler, S., Salama, H., Reingewirtz, Y. & Dubruille, J. H. (1998) Timing of loading and effect of micromotion on bone-dental implant interface: review of the experimental literature. *Journal of Biomedical Material Research* **43**, 192–203.
- Turner, C. H. (1998) Three rules for bone adaptation to mechanical stimuli. *Bone* **23**, 399–407.
- Vandamme, K., Duyck, J., Geris, L., Van Oosterwyck, H., De Cooman, M., Vander Sloten, J., Puers, R. & Naert, I. (2007a) The influence of mechanical parameters on appositional bone formation and de novo bone formation around loaded implants in the bone chamber model. An experimental study in the rabbit. *Clinical Oral Implants Research* **18**, 471–480.
- Vandamme, K., Naert, I., Geris, L., Van Oosterwyck, H., Vander Sloten, J., Puers, R. & Duyck, J. (2007b) The influence of controlled immediate loading and implant design on peri-implant bone formation. *Journal of Clinical Periodontology* **34**, 172–181.
- Vandamme, K., Naert, I., Vander Sloten, J., Puers, R. & Duyck, J. (2007c) Effect of implant surface roughness and loading on peri-implant bone formation. *Journal of Periodontology* (in press).
- Verbeke, G. & Molenberghs, G. (2000) *Linear Mixed Models for Longitudinal Data*. New York: Springer-Verlag.
- Verbeke, G. & Molenberghs, G. (2005) *Models for Discrete Longitudinal Data*. New York: Springer-Verlag.
- Wennerberg, A., Albrektsson, T., Andersson, B. & Krol, J. J. (1995) A histomorphometric and removal torque study of screw-shaped titanium implants with three different surface topographies. *Clin Oral Implants Res* **6**, 24–30.
- Wiskott, H. W. & Belser, U. C. (1999) Lack of integration of smooth titanium surfaces: a working hypothesis based on strains generated in the surrounding bone. *Clinical Oral Implants Research* **10**, 429–444.
- Wolfinger, G. J., Balshi, T. J. & Rangert, B. (2003) Immediate functional loading of Brånemark system implants in edentulous mandibles: clinical report of the results of developmental and simplified protocols. *International Journal of Oral and Maxillofacial Implants* **18**, 250–257.

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**Clinical Relevance**

*Scientific rationale for the study:* Mechanical loading as well as implant surface roughness are known to affect peri-implant bone behaviour. This study was designed to investigate their interactive impact on the bone healing processes.

*Principal findings:* Implant roughening had no significant effect in the absence of loading. Mechanical loading stimulated peri-implant bone, although this was only significant in case of surface roughening.

*Practical implications:* Mechanical loading might indeed stimulate peri-

implant bone formation, provided restricted interfacial micromotion and an optimal force transfer from the implant towards the surrounding tissues which can be facilitated by surface roughening.

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