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Influence of whole-body vibration time on peri-implant bone healing: a histomorphometrical animal study

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Abstract

Purpose: To examine the influence of time of low-magnitude, high-frequency (LMHF) loading, whole-body vibration (WBV) on peri-implant bone healing.

Materials and Methods: A custom-made Ti implant was inserted into the medio-proximal site of one tibia of 95 rats and was left to heal for 1 or 4 weeks. The daily WBV consisted of 15 consecutive frequency steps (12, 20, 30, . . . , 150 Hz) at an acceleration of 0.3 *g*. The rats were divided into five groups with different loading times: 0 (control/non-loading), 1.25, 2.5, 5 and twice 1.25 min. (with an interim recovery period) of loading. Bone-to-implant contact (BIC) and peri-implant bone fraction were measured.

Results: BIC of every test group was significantly higher than that of the control group for both healing periods. In the 4-week healing group, BIC and BFs (in all region of interests) were significantly higher in the case of twice 1.25 min. of loading compared with 1.25 min. of loading.

Conclusion: Time of loading significantly influenced the effect of the WBV on peri-implant bone healing. Twice 1.25 min. of loading appears to have the most favourable effect. LMHF loading with a particular time sequence can stimulate peri-implant bone healing and formation.

Key words: high-frequency loading; low-magnitude loading; oral implant; peri-implant bone; rat tibia; time; whole-body vibration

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Many studies have already confirmed that low-magnitude, high-frequency (LMHF) loading can stimulate bone healing and bone formation (Rubin et al. 2001a, b, Omar et al. 2008, Goodship et al. 2009, Hwang et al. 2009, Judex et al. 2009, Sehmisch et al. 2009, Shi et al. 2010). This has been applied clinically as a non-pharmacological intervention in the treatment of osteoporosis (Rubin et al. 2004, 2006, Verschueren et al. 2004, Ward et al. 2004, Gilsanz et al. 2006). Specific loading parameters, such as frequency, magnitude and loading duration, seem to play a role in the impact of LMHF loading on bone (Rubin & McLeod 1994, Oxlund et al. 2003, Castillo et al. 2006, Judex et al. 2007, Rubinacci et al. 2008).

The process of osseointegration of oral implants is similar to bone healing (Berglundh et al. 2003, Mavrogenis et al. 2009). Therefore, it was speculated that LMHF loading might positively affect peri-implant bone healing and osseointegration as well. A pilot study was therefore performed to test the stimulating potential of LMHF in a rat tibia model by means of whole-body vibration (WBV) (Ogawa et al. 2010). In this study, an osteogenic response was observed and therefore a positive effect on peri-implant bone healing and osseointegration. This was also confirmed by Akca et al. (2007), who reported a similar response around implants in ovariectomized rats after a 14-day healing period, based on microCT analysis. These results confirm the osteogenic potential of LMHF loading also around titanium implants.

As mentioned above, loading parameters such as frequency, magnitude

Conflict of interest and source of funding statement

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and loading duration affect the osteogenic impact of LMHF loading (Rubin & McLeod 1994, Oxlund et al. 2003, Castillo et al. 2006, Judex et al. 2007). The relative contribution of the individual parameters, however, is still unclear.

Concerning the effect of loading time on bone, it is known that the bone stimulation increases with the load duration until a certain saturation point is reached (Burr et al. 2002, Robling et al. 2002, Srinivasan et al. 2002). Furthermore, not only the load duration as such but also the insertion of a rest or recovery period seems to play an important role (Robling et al. 2000, 2001, 2002, Umemura et al. 2002). Robling et al. (2000) found that the osteogenic potential of loading increased when the loading was applied intermittently, i.e. three (four times 90 load cycles) or five (six times 60 load cycles) rest periods in between loading cycles led to a higher bone stimulating effect, compared with no (360 cycles at once) or just one (two times 180 load cycles) rest period. Also, the duration of the rest period is decisive. Robling et al. (2001) found a rest period of 4–8 h to be most efficient. This rest period has been suggested to lead to an optimal bone response because it allows recovery of the cell mechanosensitivity, the cellular communication and the bone fluid flow (Gross et al. 2004, Judex et al. 2009).

This study aims to investigate the effect of LMHF loading duration and sequence on peri-implant bone healing and osseointegration, as this has not been studied previously. It was hypothesized that the osteogenic response increases with increasing load duration and with the inclusion of a rest period.

Materials and Methods

Animals and surgical procedure

Ninety-five male Wistar rats (3 months old) with an average weight of 353.7 g (SD ± 12.9) were used in this study. Custom-made cylindrical screw-type implants (∅2 × 10 mm) were obtained from a titanium rod (99.6% Ti, Goodfellow Cambridge Ltd., Huntingdon, UK) (Fig. 1a). Before use, the implants were cleaned in an ultrasonic bath and decontaminated with a mixture of HF (4%) and HNO_3 (20%). The use of HF and HNO_3 etched the implant surface, resulting in an R_a value of 0.45 μm, which was determined using a scanning white-light interferometer (Wyko NT 3300; Veeco Metrology Inc., Tucson, AZ, USA), and sterilized using an autoclave. Implants were inserted into the medio-proximal site of one of both tibiae (Fig. 1b).

The rats were anaesthetized with 2.5% Isoflurane (Isoflurane USP®, Halocarbon, NJ, USA). Both cortices were perforated with a low rotational speed under constant saline cooling. To achieve good primary stability, a surgical drill 0.3 mm smaller than the implant diameter was used. After manual implant insertion by means of a custom-fit wrench, the wound was closed by resorbable sutures (Vicryl® 3-0, Ethicon, Somerville, NJ, USA). The animals were sacrificed by cervical displacement under isoflurane-induced anaesthesia, 1 and 4 weeks after implant installation.

The research protocol was approved by the local ethical committee for laboratory animal research of the Katholieke Universiteit Leuven (P029/2008) and was performed according to the Belgian animal welfare regulations and guidelines.

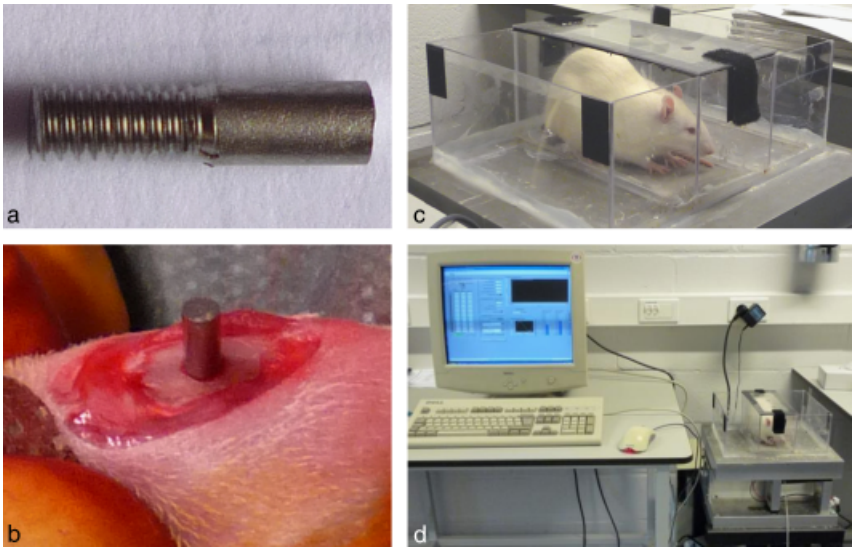

Fig. 1. (a) Custom-made titanium implant, (b) implant inserted into the medio-proximal site of the tibia, (c and d) custom-made WBV device.

Table 1. Loading protocol

Group	Loading time (min.)	Frequency (Hz)	Acceleration (*g*)
CTR	0	12–150	0.3
Test 1	5 20 s per each frequency	12–150	0.3
Test 2	2.5 10 s per each frequency	12–150	0.3
Test 3	1.25 5 s per each frequency	12–150	0.3
Test 4	1.25 × 2 5 s per each frequency × 2	12–150	0.3

Each healing period (1 and 4 weeks) is subdivided into five loading groups [a control group with no loading, and groups with 5, 2.5, 1.25 and twice 1.25 min. (interval of 4 h) loading].

WBV and loading protocol

The LMHF loading was applied through WBV. A custom-made WBV device was used (Department of Mechanical Engineering, Division of Biomechanics and Engineering Design, K. U. Leuven) (Fig. 1c and d).

The animals were randomly divided into two groups of different healing times. In one group ($n = 45$ animals), the implants healed for 1 week, whereas the implants in the other group ($n = 50$) healed for 4 weeks. Each group was subdivided into five groups with different loading times: a control group (no loading) and four test groups with 1.25, 2.5, 5 and twice 1.25 min. (interval of 4 h) of loading. The vibration started the next day after surgery. The daily vibration consisted of 15 consecutive frequency steps (12, 20, 30, 40, . . . , 150 Hz), applied in a randomized way, and all with a 0.3 *g* acceleration. Table 1 shows the loading scheme.

Processing of the samples

After sacrificing the animals, the tibia with the implant was immediately fixated in a $CaCO_3$-buffered formalin solution and dehydrated in increasing concentrations of alcohol. After dehydration, the samples were embedded in polymethylmethacrylate. The embedded samples were cut by a diamond saw (Leica SP 1600, Leica Microsystems, Nussloch, Germany) along the axis of the tibia and implant.

After polishing to a final sample thickness of 20–30 μm (Exakt 400 CS, Exakt Technologies Inc., Norderstedt, Germany), the sections were stained with Von Giesen's picrofuchsin red to visualize the mineralized bone tissue and with Stevenel's blue to visualize the non-mineralized tissue.

Histomorphometrical analysis

The histological and histomorphometrical analyses were performed using a light microscope with a magnification of $\times$ 100 (Leica Laborlux, Wetzlar, Germany). The samples were scanned using a high-sensitivity video camera (AxioCam Mrc5, Zeiss, Göttingen, Germany). The histomorphometrical analyses were performed using the image-analysing software package (Axiovision 4.0, Zeiss) and customized scripts for semi-automatic analyses (Ogawa et al. 2010). The reproducibility of the measurements was checked by evaluation of the differences of two examiners. As this inter-examiner variation was limited, one examiner continued with the rest of the measurements.

The following analyses were performed:

(i) Bone-to-implant contact (BIC) = (summation of the lengths of all contact regions between bone and implant (μm)/total length along the implant from first to last BIC (μm)) $\times$ 100.
(ii) Bone fraction (BF) = (area occupied by bone (μm^2)/reference area (μm^2)) $\times$ 100.

Three reference areas were defined and included the peri-implant tissues of both cervical (upper) and apical (lower) cortex as well as the medullar cavity. The peri-implant reference sites differed in the distance from the implant. The region of interest (ROI) 1 ranged from 0 (implant surface) to 100 μm, from 100 to 500 μm (ROI2) and from 500 to 1000 μm (ROI3) (Fig. 2).

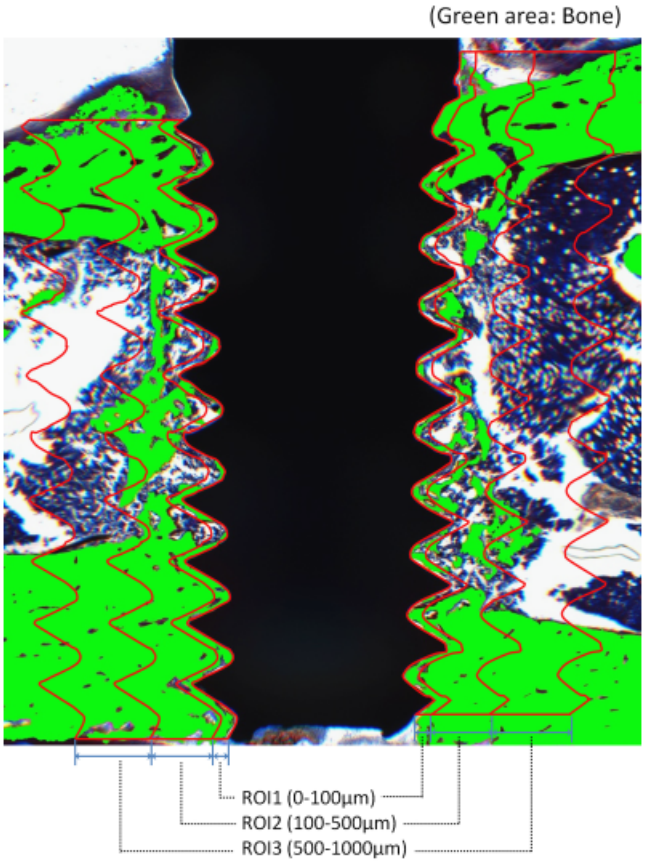

Fig. 2. Bone fraction analysis. The amount of bone in three different regions of interest (ROI) is measured. These regions differ in their distance to the implant. ROI1: 0–100 μm, ROI2: 100–500 μm and ROI3: 500–1000 μm.

BIC and BF measurements were performed at both medial and distal implant sites.

Statistical analysis

Two-way analysis of variance (ANOVA) and the Tukey HSD test were performed to evaluate differences between the two healing periods and the different loading groups (SPSS ver. 13.0, Chicago, IL, USA). The significance level of $p < 0.05$ was used.

Results

In the 4-week healing period, three samples were excluded from histomorphometrical analyses because of skin infection or technical failure during sample processing.

Histological observations

Figure 3 shows a representative example of a loaded implant that was allowed to heal for 1 or 4 weeks. After 1 week of healing, a clear osteogenic response was observed. After 4 weeks of healing, the immature bone around the implant reorganized and became much denser.

BIC analysis

In Fig. 4a, BICs of the two healing periods are shown. BIC was significantly influenced by the loading time as well as by the healing period (ANOVA; $p < 0.01$). A subsequent post hoc analysis indicated that the BIC of each test group was significantly higher than that of the control group in both the 1- and the 4-week healing periods (Tukey's HSD test; $p < 0.05$ or $p < 0.01$). Moreover, in the 4-week healing period, BIC was significantly higher in the twice 1.25 min. loading group compared with the 1.25 min. loading group (Tukey's HSD test; $p < 0.05$).

BF analysis

BF at 0–100 µm (ROI1)

Although no significant difference in BF was observed between the two healing periods (ANOVA; $p > 0.05$), BF was sig-

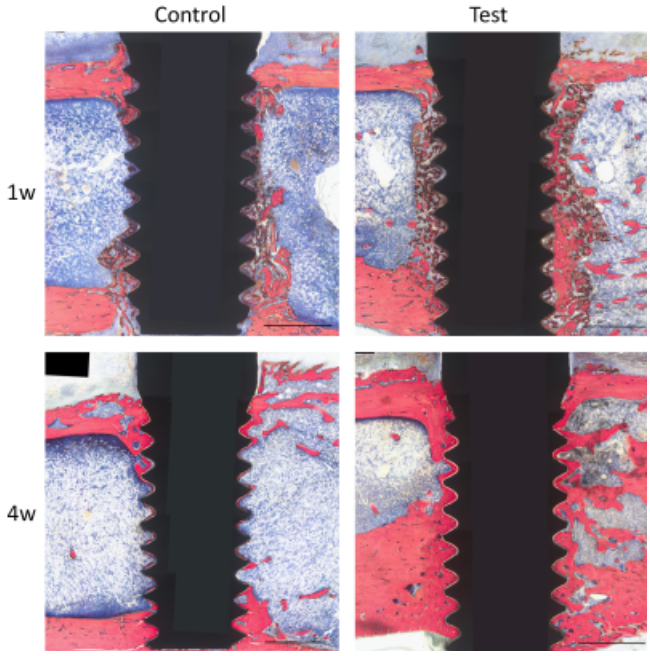

Fig. 3. Representative images of the test and control group from the 1-week healing period (a) and the 4-week healing period (b). Scale bars: 1 mm.

nificantly influenced by the loading time (ANOVA; $p<0.01$) (Fig. 4b). In the 1-week healing period, it was found that the test groups, except for the 2.5 min. loading group, showed a significantly higher BF than the control group (Tukey's HSD test; $p<0.05$ or $p<0.01$). In the 4-week healing group, a significant difference was found between the control and the 5 min. loading group (Tukey's HSD test; $p<0.05$) and also between the control and the twice 1.25 min. loading group (Tukey's HSD test; $p<0.01$). Moreover, BF of the twice 1.25 min. loading group was significantly higher than both of the 1.25 min. and the 2.5 min. loading groups (Tukey's HSD test; $p<0.05$).

BF at 100–500 µm (ROI2)

BF (ROI2) was significantly influenced by the loading time as well as by the healing period (ANOVA; $p<0.01$). In the 4-week healing period, a statistical difference between the twice 1.25 min. loading group and the control group, as well as between the twice 1.25 min. and the 1.25 min. loading group, was found (Tukey's HSD test; $p<0.05$) (Fig. 4c).

BF at 500–1000 µm (ROI3)

Although no statistical difference in BF was observed between the two healing periods, BF was significantly influenced by the loading time (ANOVA; $p<0.01$) (Fig. 4d).

For the 4-week healing period, the BF of the twice 1.25 min. loading group is statistically higher than the control group and the other loading groups, except for the 2.5 min. loading group (Tukey's HSD test; $p<0.05$ or $p<0.01$).

Discussion

Based on our pilot study, which found a positive effect of the LMHF loading on peri-implant bone (Ogawa et al. 2010), here we further investigated the influence of time factors on the impact of the LMHF loading on peri-implant bone. It was hypothesized that these time factors do play a role on peri-implant bone healing and osseointegration.

The results of this study confirm that both BIC and BF are significantly influenced by LMHF loading. This was also observed by other researchers in bone with (Akca et al. 2007) or without implants (Rubin et al. 2001a, b, Omar et al. 2008, Goodship et al. 2009, Hwang et al. 2009, Judex et al. 2009, Sehmisch et al. 2009, Shi et al. 2010).

To test our hypothesis, the loading group was subdivided into four groups with different loading durations and with or without a recovery period. The overall results of the post hoc analysis (Tukey's HSD test) clearly showed that the twice 1.25 min. loading group exhibited the most pronounced stimulating effect on BIC and BF in all three ROIs after 4 weeks of healing. The positive influence of the twice 1.25 min. loading can be explained by the inclusion of a rest period between two loading sessions per day. Robling et al. (2000, 2002) already indicated the beneficial effect of such rest periods on loading-induced bone formation. They found that division of a given bout of loading cycles into several loading sessions could lead to an increase of the bone response to the mechanical intervention. This rest period seems to allow an optimal recovery of the cell's mechanosensitivity, which was believed to decrease along with loading due to bone cells possessing the ability to accommodate their physical and biological environment (Turner, 1999). As proposed by Robling et al. (2000), the loss of mechanical sensitivity, and subsequent resensitization following a load-free recovery period, is mediated in part by the actin cytoskeleton in bone cells.

Not just the insertion of a rest period but also the duration of this rest period is important for the positive effect on BIC and BF. This rest period was 4 h in the current study. Some researchers investigated the optimal recovery period of mechanosensitivity in more detail (Robling et al. 2001, Umemura et al. 2002). Robling et al. (2001) reported that a rest period as short as 10 s after each loading cycle within a bout can transform an otherwise ineffective loading regime into a highly osteogenic stimulus. Furthermore, it was shown that a rest period of 4 h presented a significantly improved osteogenic effect than the no-rest group (Robling et al. 2001). This positive effect of the 4 h rest-time between loading sessions on the loading-induced bone response might offer a reasonable explanation for the findings in our study.

Regarding the loading duration, no significant differences were observed among the 5, 2.5 and 1.25 min. loading groups. Nevertheless, several studies (Rubin & Lanyon 1984, Turner et al. 1994, Umemura et al. 1997) indicated that increasing the duration of loading significantly increased the bone formation rate and the bone mineral content. However, it was also reported that after a certain threshold, saturation occurs and results in a plateau (Rubin & Lanyon 1984, Umemura et al. 1997, Burr et al. 2002). This might be an explanation for

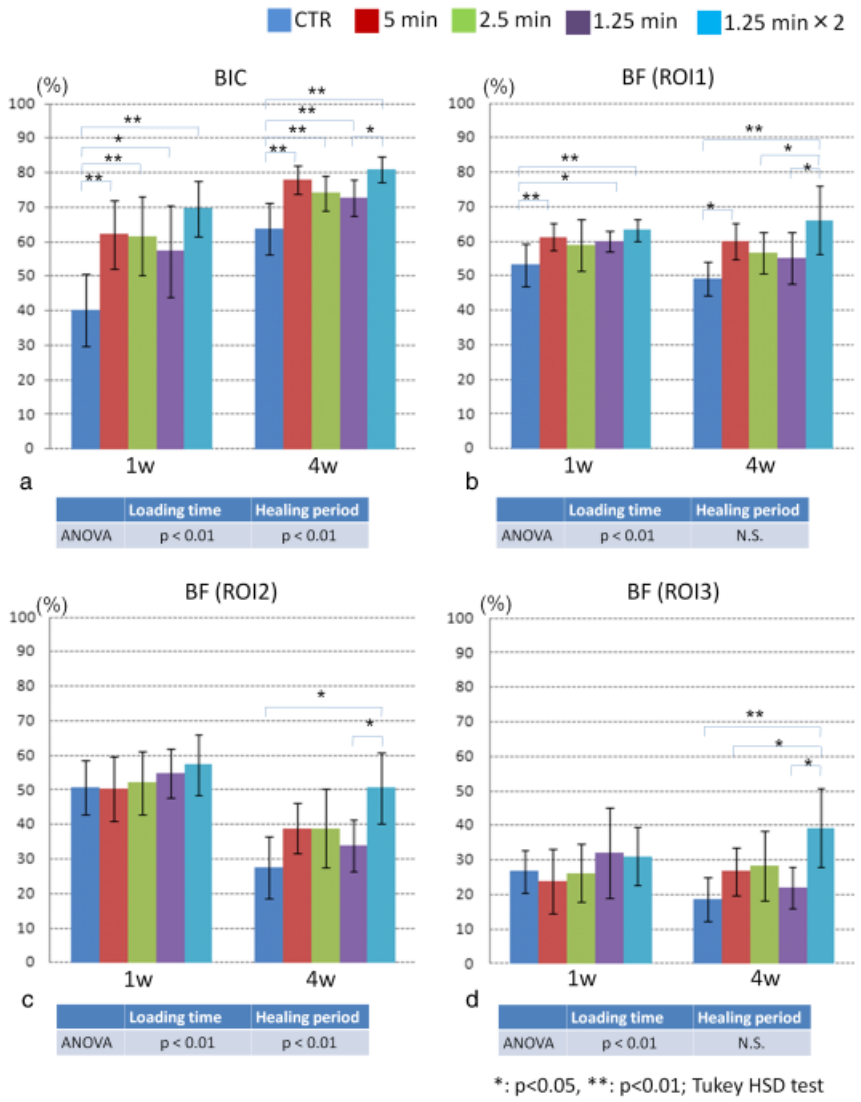

Fig. 4. Histomorphometrical results of the bone-to-implant contact (BIC) and bone fraction (BF). The graphs show the means and standard deviations of the BIC (a) and BF (b–d) for the different healing periods (Tukey's HSD test; $^{*}p<0.05$, $^{**}p<0.01$).

the lack of a significant difference among the 5, 2.5 and 1.25 min. loading groups.

Interestingly, the results of the current as well as our previous study (Ogawa et al. 2010) reveal that the loading effect seems to be more distinct in the area closest to the implant (BIC and BF of ROI1). There are three possible reasons to explain for this. The first is that differentiating tissues react better to loading than non-differentiating tissues in a condition such as bone healing (Omar et al. 2008, Goodship et al. 2009, Shi et al. 2010). As the differentiating tissues are mainly located at the interface, the loading effect is supposed to be more distinct in the neighbouring region. Secondly, there could be an additional loading-related influence on peri-implant bone healing and osseointegration because of the interface between the titanium implant and its surrounding bone. Because of the different material properties of titanium and bone, these materials will behave differently to the loading, thereby creating a certain mechanical environment at the interface, which is likely to differ from the rest of the bone (Duyck et al. 2007, Vandamme et al. 2007). This particular mechanical situation might be responsible for a different kind of cell triggering and deposition of, e.g. ions such as calcium and phosphate ions (Ho & Melbin, 2005), which may also contribute to faster osseointegration. Finally, there is the combination of the two previously mentioned reasons. However, these mentioned possibilities need further investigation.

Clear differences were observed between the results after 1 *versus* 4 weeks. BIC was significantly higher after 4 weeks of healing. This observation can probably be explained by the process of osseointegration. After 1 week, BIC can be established by bone apposition from the surrounding bone in those implant regions that were initially not in direct contact with the bone. In the areas where the bone was in direct contact with the implant, a process of bone resorption needs to precede the bone apposition (Botticelli et al. 2003, 2005). This implies that there is a phase of decreasing BIC before the implant becomes biologically integrated. After 1 week of healing, considerable woven bone formation is observed in the medullar area. After 4 weeks, this newly formed bone decreased in volume and has rearranged into denser and better organized bone, often in contact with the implant.

Close to the implant, there is considerable bone at both healing periods. Rather immature bone is seen after 1 week of healing, whereas more organized bone is seen after 4 weeks of healing. Therefore, a similar BF in ROI1 is seen for both healing periods (ANOVA; $p>0.05$). BF in ROI2, on the other hand, is significantly higher for the 1-week healing period. This can again be explained by the massive osteogenic reaction in the medullar area, whereby considerable immature bone is formed next to the implant and stretches relatively away from the implant. After 4 weeks of healing, this immature bone has rearranged and is more concentrated around the implant, resulting in a lower BF in this area for this healing period. For the BF at ROI3, no significant difference was found between the 1- and the 4-week healing periods. At this distance from the implant, almost no immature bone was found in the 1-week healing period. This is possibly the reason why no difference was found between the two healing periods. However, even in this area, a significant influence of the loading was found (ANOVA; $p<0.05$). This result might reflect the accelerating effect of the pure osteogenic stimulus of loading to the mature bone.

As mentioned before, besides time factors, there are many more loading parameters such as magnitude or frequency that affect the loading effect. These factors, as well as their interaction, should be investigated in order to establish an optimal bone-stimulating loading regime to enhance peri-implant bone healing and osseointegration.

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Clinical Relevance

Scientific rationale for study: Recent studies provided evidence that low-magnitude, high-frequency loading has an osteogenic effect that depends on the loading parameters used. The present study investigated the influence of time factors, such as load duration and rest periods, on the impact of LMHF loading on peri-implant bone healing and osseointegration.

Principal findings: Time factors do play a role in that a rest period improves the loading effect.

Practical implications: An appropriate LMHF loading can improve and accelerate peri-implant bone healing and osseointegration.