Early cortical bone healing around loaded titanium implants: a histological study in the rabbit

Elke Slaets
Ignace Naert
Geert Carmeliet
Joke Duyck

Authors’ affiliations:
Elke Slaets, Ignace Naert, Joke Duyck, Department of Prosthetic Dentistry, BIOMAT Research Group, K.U., Leuven, Belgium
Geert Carmeliet, Department of Experimental Medicine, Laboratory of Experimental Medicine and Endocrinology, K.U., Leuven, Belgium

Correspondence to:
Joke Duyck
Department of Prosthetic Dentistry/BIOMAT Research Group
School of Dentistry, Oral Pathology and Maxillofacial Surgery
Faculty of Medicine, Catholic University of Leuven [K.U. Leuven]
Kapucijnenvoer 7
Leuven 3000
Belgium
Tel.: +32 16 33 24 68
Fax: +32 16 33 23 09
E-mail: Joke.Duyck@med.kuleuven.be

Key words: histology, mechanical loading, oral implant, rabbit

Abstract
Objectives: To identify the role of immediate implant loading on the early phases of the bone healing responses.

Material and methods: Implants were placed in rabbit tibial diaphyses and left to heal for 3, 7, 14, 28 or 42 days. Half of the animals received an immediate loading protocol of 2.2 N at 3 Hz for 1800 cycles and 5 days/week, whereas the others served as unloaded controls. Histological assessment was combined with histomorphometrical measurements.

Results: At early time-points, an endosteal and periosteal new bone formation was found, while the cortex itself contained damaged osteocytes. At later time-points, new bone formation was also found at the cortical level itself. Differences between groups were found mainly in this new bone formation process, with larger reactions for the endosteal and periosteal bone in the loaded group after 28 and 42 days, respectively. At the end-point of the experiment, bone formation at the cortical level was reduced in the loaded group compared with the control group.

Conclusions: These results show that the immediate loading protocol caused no differences in the sequential events leading to osseointegration in cortical bone. However, the processes of new bone formation originating from the endosteum and the periosteum lasted longer compared with the unloaded controls.

As the first reports on osseointegration [Bränemark et al. 1969], a standard healing period of at least 3 months in the mandible and 6 months in the maxilla has been suggested before implant loading [Brånemark et al. 1977; Adell et al. 1981; Zarb & Jansson 1985; Albrektsson et al. 1986]. The rationale for choosing this delayed loading protocol is, among others, based on several early reports indicating that premature loading increased the risk of fibrous tissue encapsulation of the implants rather than forming direct bone apposition [Brunski et al. 1979; Akagawa et al. 1986; Maniatopoulos et al. 1986]. However, recent studies have pointed out that immediate loading per se is not necessarily responsible for this fibrous encapsulation. More likely, surpassing a threshold of implant micromotion during the healing phase interferes with bone repair around the implant [Szmukler-Moncler et al. 1998; Duyck et al. 2006].

Because of improvements made in surgical techniques, implant macro- and microtopography and for the sake of the patient benefits (shortening of treatment duration) a tendency towards early and immediate loading is observed in the clinic today. Some recent studies showed comparable high success rates for these loading protocols, both in the clinical setting [Randow...
et al. 1999; Testori et al. 2004), as in experimental animal studies. In the latter, studies have been performed with implants placed in the oral cavity, e.g., of dogs [Schultes & Guggl 2001; Berglundh et al. 2003; Abrahamsson et al. 2004], minipigs [Meyer et al. 2003, 2004; Nkenke et al. 2003, 2005] or monkeys [Piattelli et al. 1998]. Other experimental animal studies used the animals’ long bones, enabling for example the application of a very controlled loading protocol [De Smet et al. 2006; Duyck et al. 2006, 2007; Vandamme et al. 2007a, 2008].

However, limited information on the effect of loading during the early time-points of the bone healing process is available today. In previous studies, we investigated the early phases of bone healing around unloaded titanium implants, both in the cortical and trabecular bone of the rabbit tibia [Slaets et al. 2006, 2007]. These studies showed that the insertion of an implant initiated a series of biological processes including the formation of a hematoma and altered nuclear morphology of osteocytes surrounding the implantation site at an early time-point. This was followed by intensive bone remodeling and the formation of new bone leading to the osseointegration of the implant. The manifestation and duration of these processes were dependent on the type of bone considered.

In the present study, we aimed to more specifically characterize the impact of immediate loading on these early healing processes around implants, in order to elucidate the differences between the standard [unloaded healing of implant] protocol and the immediate loading protocol used in the clinic. Therefore, bone healing and adaptation around titanium screw-shaped loaded and unloaded implants, placed in the same rabbit cortical tibia model as mentioned above, were examined. The tissue response to the implant insertion was investigated as well as the effect of immediate implant loading.

Materials and methods

Animals and implants

Twelve mature New Zealand White rabbits were used and divided in two even groups: a loaded [test] and an unloaded [control] group. Sixty Ti-6Al-4V one-piece percutaneous implants [AstraTech, Mölndal, Sweden] with dimensions of 10.5 × 1.8 mm were used. The endosteous part was screw-shaped and TiO₂-blasted (Ra-value: 1.74 μm). Only the percutaneous part was turned and designed to fit a mechanical stimulation lever.

Surgical procedure

Five implants were placed bi-cortically in the diaphyses of the rabbit tibiae, with an interval of 25 mm between consecutive implants. Implant site selection was divided over both diaphyses and randomized before implant surgery. Insertion of the five implants was performed using an inverse time schedule, resulting in healing times of 3, 7, 14, 28 and 42 days at the time of animal sacrifice.

The animals were pre-anesthetized by an intramuscular injection of ketamin [25 mg/kg, Ketamine 1000 CEVA; CEVA Sante Animale, Brussels, Belgium] and xylazin [5 mg/kg, Vexylan, Ceva Sante Animale, Brussels, Belgium]. The anesthetia was given by intravenous injection of propofol [Diprivan 1%, AstraZeneca, Brussels, Belgium, dose of 8 ml/h]. A small skin incision was made to provide access to the bone. Implantation sites were prepared by drilling a cavity with low rotational speed until a diameter of 1.75 mm, while diffuse irrigation with sterile physiological saline was maintained. Implants were inserted by manual torque. Postoperatively, the animals were given an intramuscular injection of buprenorphin [0.05 mg/kg, Temgesic; Reckitt and Colman, Richmond, VA, USA] as analgesic and antibiotics [0.05 mg/kg, Benzilpenicillina Potassica; Biopharma, Piedmont, Italy] for 3 and 5 days, respectively. At the end of the experiment, the animals were sacrificed with an overdose of embutramide, mebenzoniumiodide and tetracaine hydrochloride [T61; Intervet, Unterschleissheim, Germany]. The study protocol was approved by the Ethical Committee on Animal Testing of the Catholic University of Leuven, Belgium.

Loading protocol

Mechanical stimulation started 1 day after implant installation and followed the same set-up as previously reported [De Smet et al. 2005, 2006]. In short, a sinusoidally varying bending moment was applied with a force-controlled electro-mechanical shaker, throughout a lever connected onto the implant. The horizontal lever was screw-retained onto the implant, in alignment with the long axis of the tibia. During stimulation, the tibia was firmly fixed by an alginate casting to the base plate of the stimulator to ensure reproducible mechanical stimulation. In the present study, test implants were stimulated at 2.2 N with a frequency of 3 Hz for 1800 cycles, for 5 day/week. In addition, control implants were daily manipulated including the connection of the lever but without applying any load [sham]. The strain rates applied on the loaded samples were based on a previous study from our group [De Smet et al. 2006], indicating comparable parameters as osteogenic. Cadaver strain gauge measurements indicated that these parameters amounted 1000 με/s at 2.2 N [data not shown]. To this end, the strain gauge was glued on the outer surface of the tibial bone in the direction of the stimulation lever arm, at 1 mm distance from the implant surface as the grid dimensions of the strain gauge prevented closer contact.

Processing of bone samples

Immediately after animal sacrifice, tibial specimens were isolated and fixed in 2% paraformaldehyde. The bone segments were decalcified in 0.5 M EDTA [pH 7.4]/PBS at 4°C and paraffin-embedded, whereupon the implants were gently unscrewed. Next, the samples were re-embedded and 4 μm sections were made [HM360; Microm, Walldorf, Germany] parallel to the long axis of the tibial bone and the implant. Sections were stained with hematoxyline-eosine [H–E] for general morphological analysis. Osteoclasts were visualized by staining for tartrate-resistant acid phosphatase [TRAP] activity, whereas the endothelial cells of the blood vessels were visualized by CD31 immunohistochemical staining [DAKO, Glostrup, Denmark]. Cbfα1 staining was performed to identify the osteogenic cells [Sasaguri et al. 2002; Brönckers et al. 2003, 2005].

Histomorphometric analysis

Histomorphometry was conducted using an image analyzing system [KS400 V3.00; Kontron Electronik, Munich, Germany]. Three sections per tissue sample were analyzed and data were averaged. The following histomorphometric measurements were performed on the H–E stained sections.
A region of osteocytic cell damage (visualized as a loss of nuclear staining in the H–E stained sections) was assessed by quantifying the distance between the implant cavity and the border of this region at three pre-defined depths in the cortical bone. Results were averaged for the upper (site of lever attachment) and lower cortex (Fig. 1a). To assess bone remodeling, the area occupied by basic multicellular units (BMUs) was calculated relative to a reference area of 500 µm adjacent to the implant cavity. The BMUs were defined as organized structures of multiple osteoclasts and osteoblasts together with blood vessels. The area of these structures was manually depicted on the scanned images, quantified per cortex and expressed as the percentage of the total bone area in the considered region (Fig. 1a). The formation of new bone at the cortex was quantified as the average distance in which new bone is found, measured per indentation. Again, the results were averaged per cortex (Fig. 1b). The endosteal new bone formation was quantified by measuring the total area involved and expressed as percentage of a control area, the latter defined over a distance of 2500 µm from the implant cavity (Fig. 1b). Simultaneously, the bone density of the endosteal new bone formation was measured after 3 and 42 days of healing. It was defined as the area of the newly formed bone matrix relative to the area of the bony tissue (Fig. 1b). Finally, the thickness of bone apposition by the periosteal new bone formation was quantified adjacent to the implant (Fig. 1b).

**Statistical analysis**

Differences between the different time-points were tested by repeated-measures ANOVA (GraphPad Instat, San Diego, CA, USA) followed by a Tukey post-test. The level of significance was set at $\alpha = 0.05$. The data are expressed as means, with an indication of the standard errors of the mean (SEM).

**Results**

Fifty-four samples healed uneventful whereas six samples showed signs of infection at the percutaneous site. This inflammation was evident on the histological sections and these samples were therefore excluded from the data set. No difference was found in the occurrence of infection between the test and control group. An increase in infection rate was observed with later time-points as compared with the earlier time-points (Table 1).

### Table 1. Number of infected samples per time point and per condition

<table>
<thead>
<tr>
<th>Time points (day)</th>
<th>Test</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>28</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>42</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

A region of osteocytic cell damage (visualized as a loss of nuclear staining in the H–E stained sections) was assessed by quantifying the distance between the implant cavity and the border of this region at three pre-defined depths in the cortical bone. Results were averaged for the upper (site of lever attachment) and lower cortex (Fig. 1a). To assess bone remodeling, the area occupied by basic multicellular units (BMUs) was calculated relative to a reference area of 500 µm adjacent to the implant cavity. The BMUs were defined as organized structures of multiple osteoclasts and osteoblasts together with blood vessels. The area of these structures was manually depicted on the scanned images, quantified per cortex and expressed as the percentage of the total bone area in the considered region (Fig. 1a). The formation of new bone at the cortex was quantified as the average distance in which new bone is found, measured per indentation. Again, the results were averaged per cortex (Fig. 1b). The endosteal new bone formation was quantified by measuring the total area involved and expressed as percentage of a control area, the latter defined over a distance of 2500 µm from the implant cavity (Fig. 1b). Simultaneously, the bone density of the endosteal new bone formation was measured after 3 and 42 days of healing. It was defined as the area of the newly formed bone matrix relative to the area of the bony tissue (Fig. 1b). Finally, the thickness of bone apposition by the periosteal new bone formation was quantified adjacent to the implant (Fig. 1b).
an indication of the osteogenic character of the cells present [Fig. 2c]. At the periosteal side, new bone formation was also noted [Fig. 2a]. After 7 days of healing, this bone formation appeared to increase compared with the earlier time-point, as could be confirmed in the histomorphometric measurements of these reactions [Fig. 3a and b].

In the first week after implant installation, an alteration in the appearance of the osteocytic lacunae was also noted. Whereas the normal appearance of the osteocytes was found at a distance of the implant, the lacunae close to the implant cavity appeared devoid of osteocytes, as the nuclear staining was absent (Fig. 2d). The histomorphometric measurement of this region indicated that a region of 300–400 μm from the implant was involved (Fig. 3c). Inside the bone marrow, shattered pieces of cortical bone were found at the early time-points, showing no signs of intact osteocytes.

After 7 days of implant installation, the TRAP staining showed the presence of osteoclasts near the implant cavity (Fig. 2e).

14 days

After 14 days of implant installation, new bone formation was noticed in the cortical bone area [Fig. 4a and b]. The collagen fibers of the new bone were laid down in a different direction compared with the pre-existing bone. Histomorphometric measurements indicated a tendency towards more new bone in the loaded samples [Fig. 3d].

At this time-point, both endosteal and periosteal new bone formation enlarged compared with previous time-points [Fig. 3a and b], the former also being responsible for the presence of a thin bone collar connecting the two cortices. A region of affected osteocytes was again noticed and appeared confined to the same region (Fig. 3c) as at the previous time-point.

In the cortical bone, an increased area of BMUs was noted in a region of 500 μm from the implant, as was validated in the measurements in this region (Fig. 3e). These BMUs were accompanied by blood vessels, as was visualized on the CD31-stained sections (Fig. 4c).

28 and 42 days (Fig. 4d)

After 28 days of healing, a significant maximum was found in the region of osteocytic cell damage in the control group (P < 0.05 vs. t = 3, 7, 42 days), as indicated in Fig. 3c, after which the affected region decreased again towards the 42 days time-point. The remodeling area also decreased at these time-points (Fig. 4d), as was indicated on the H–E, TRAP and CD31 stainings.

The region of new bone formation at the cortex showed a steady increase in both groups, with a significant maximum after 42 days in the control group (P < 0.05 vs.
Fig. 3. Overview of the results of the histomorphometric measurements. (a) Quantification of the relative surface of the endosteal reaction at the different time-points, showing a maximum in the test group after 28 days of healing ($P<0.05$ between test and control group). (b) Measurement of the periosteal reaction, indicating a sustained increase in the test group, while a maximum is found after 14 days in the control group ($P<0.05$ between test and control group). (c) Quantification of the region of osteocytic cell damage, indicating a maximum in both groups after 28 days of healing ($P<0.05$ vs. t = 3, 7, 42 days of the same group). (d) Quantification of the new bone formation at the cortices, demonstrating a steeper increase in the control group compared with the test group ($P<0.05$ vs. previous time-points of the same group). (e) Measurement of the relative surface of the basic multicellular units (BMUs) indicating a maximum in both groups after 14 days of implant installation. (f) Density measurement of the endosteal reaction indicating a significant increase at the last time-point ($P<0.05$ between indicated time-points). Parts [b–e] depict the trends in the upper (skin-protrusion) cortex. Results for the lower cortex are comparable but less distinct.
previous time-points). However, in the control group a steeper increase was found compared with the loaded test group (Fig. 3d).

The endosteal new bone formation demonstrated an additional difference between the two groups; whereas a decrease was noted in the control group, an increase was found in the test group after 28 days of healing (Fig. 3a, $P<0.05$ between test and control group). Both groups showed a decrease towards the later time-point of 42 days. At this time-point, the area occupied by the endosteal new bone formation was comparable to the area occupied after 3 days of healing. However, the density measurement of the endosteal area indicated a significant increase after 42 days of healing compared with the 3-day time-point ($P<0.05$, Fig. 3f).

Discussion

The aim of the present study was to investigate the effects of immediate implant loading on the peri-implant healing processes in cortical bone. A rabbit tibia model was used, in accordance with previous studies from our group (Slaets et al. 2006, 2007). The advantage of this model lay in the easy accessibility of the tibia for the daily loading manipulations. Loading was performed by means of a screw-retained lever, a set-up that already proved its feasibility in preceding reports from our group (De Smet et al. 2005, 2006) using a guinea-pig model. The loading protocol resulted in the transmission of a bending moment on the implant and the surrounding bone, which are also found in the clinical setting (Clelland et al. 1995; Duyck et al. 2000).

In this study, implants were removed in order to make histological sections of 4 μm, enabling a characterization of the healing and adaptive processes at a cellular level. However, the implant removal itself may have caused some interfacial damage, as indicated in our previous studies (Slaets et al. 2006, 2007). Nevertheless, this minor damage does not affect the results of this study as healing processes in the surrounding bone and marrow cavity, and not at the interface itself, were described and qualified in this study.

On the histological sections, differences between the two conditions were found, which were corroborated by histomorphometric measurements. Two processes could be distinguished, namely the impact of implant insertion on the surrounding cortical bone adaptation around loaded titanium implants.
bone and the new bone formation around the implants.

A first consequence of implant insertion was found on the osteocytic appearance. At the earliest time-point after implant installation, the first signs of osteocytic cell damage were seen as the nuclear staining of the osteocytes was lacking. This osteocytic cell damage was seen in a border around the implant. Markedly, this border was still clearly noticeable 6 weeks after implant installation.

Other studies also reported on this phenomenon [Ohtsu et al. 1997; Fujii et al. 1998; Futami et al. 2000; Ménard et al. 2000, Shirakura et al. 2003]. However, few histomorphometric measurements were performed at different time-points. In our previous study of cortical healing processes around the unloaded implant [Slaets et al. 2006], this region was quantified in a similar manner. However, in that study set-up we found a smaller region affected by the implant insertion. This difference could be explained by the different diameters of the implants used, namely 1.8 mm instead of 1 mm, leading to different stress distributions along the implant, and a more elaborate surgical protocol inherent to the broader diameter. Nevertheless, the trends found in the results of the measurement are strongly comparable, as both studies indicate a maximal region affected after 28 days, whereafter a decline is noted. When comparing the test and control group, a trend towards a higher region of cell damage around the loaded test group is found, indicating a possible additional effect of the daily immediate loading applied on these samples. A study by Eriksson et al. (1984) designated this zone of osteocytic cell damage as necrosis [or accidental cell death] caused by the surgical preparation of the bone for the implant installation. Besides, mechanical and blood vessels damage by frictional heat, leading to ischemia, were pointed out as possible etiological factors. However, in this study, an enlargement of this affected zone was seen up to 28 days after implant installation, making it unlikely to designate the surgical procedure as the only rationale for this phenomenon. This points towards a second mechanism of cell death. However, despite several efforts, no efficient staining procedures were found for the rabbit paraffin sections used in this study, leaving the second mechanism uncertain.

A second effect noted in the surrounding bone is the appearance of BMUs around the implant. These bone entities are responsible for the replacement of damaged or older bone by new bone. As it is hypothesized that a functional relationship exists between bone turnover and osteocytic cell damage [Noble et al. 1997; Boabaid et al. 2001], the appearance of these BMUs can be seen as a consequence of the osteocytic cell damage discussed above. In a previous study [Slaets et al. 2006], a comparable area measurement was performed, pointing to an elevated presence in the current study. This could be explained by the larger region of affected bone found in these experiments. No clear differences between test and control group were found in this second effect.

The process of new bone formation around the implant was first represented by the reactions of the endosteum and the periosteum, both containing osteogenic cells. After 3 days, clear bone-forming reactions on both surfaces were visualized and quantified. These can be viewed as the first reactions of the bone in response to the implant installation. The Cbfa1 staining that was performed, showed the presence of cbfa1 protein in the cells present in these reactions, indicative of the pre-osteoblastic or osteoblastic nature of these cells. For the control implants, the same trend can be seen in the histomorphometric measurements of the endosteal and periosteal reaction, with a maximum after 14 days of healing. The test implants on the other hand showed a maximum in the endosteal bone formation after 28 days of healing, after which a decline was noted. In general, a trend towards a larger endosteal reaction can be seen at the later time-points in the test group, indicating a higher degree of fixation at this level. The density measurement of the endosteal new bone formation indicated that although similar surfaces were occupied after 3 and 42 days, the bone was much denser at the later time-point, demonstrating a maturation of the bone and consequently an improved fixation of the implant in the surrounding bone. Because of this gradual densification of the endosteal bone adjacent to the cortex, it is believed that the newly formed bone itself was reinforced, thereby allowing a volume reduction in time.

When comparing the results for the measurement of the periosteal new bone formation in the two groups, another difference is found, as this reaction continued to increase towards the final time-point in the test group. This finding is again indicative of a higher degree of fixation in the test group at the periosteal level.

After 14 days of healing, a third process of new bone formation was visualized, namely at the cortical level. This cortical bone was formed to replace the affected bone, which was [in part] resorbed by the action of the osteoclasts at the early time-points. The test group showed a higher amount of new bone after 14 days of healing compared with the control group. However, due to a faster increase thereafter in the latter, this group had a higher amount of new bone formed at the 42 days time-point. The described time frame of new bone formation is in agreement with reports from other groups using the same model [Piattelli et al. 1995; Dhert et al. 1998] and with our previous report on rabbit cortical bone healing [Slaets et al. 2006], where new bone was also seen after 14 days of healing.

These results suggest that at the later time-points, the loading protocol induced less new bone formed at the cortical level, compared with the control group. However, by an increased endosteal and periosteal new bone formation, the bone appeared to provide a comparable amount of fixation for the implant, thereby demonstrating the good adaptation possibilities of bone.

Similar to these findings of an increased endosteal reaction in the loaded group, other studies from our group reported on comparable observations. In a bi-cortical guinea-pig model, De Smet et al. (2003) investigated the effect of early [after 7 days] mechanical loading on the establishment of osseointegration by means of resonance frequency analysis. While a decrease in implant stability was found for the control implants, the test implants had a progressive increase in stability over time. In the following studies [De Smet et al. 2006, 2007] it was observed that the bone mass in the peri-implant marrow cavity was significantly higher around the loaded implants, indicative of an improved bone forming reaction in the marrow cavity. This was also detected and measured in the present experiment. Where the studies...
of De Smet et al. [2007] only included one time-point for histological analysis, the present study made use of several time-points in order to mark the onset of differences found between test and control groups.

Other studies from our group using a repeated sampling bone chamber methodology with a central implant installed in the tibia of rabbits, also reported on a loading-related bone response [Vandamme et al. 2007a, 2007b], indicative of a bone-stimulating effect induced by the mechanical loading.

When extrapolating our results to the clinical situation, several points should be taken into account. Firstly, this study was performed in tibial cortical bone, where the jawbones consist of two different types of bone, namely cortical and trabecular bone. While the former is mainly found in the mandible, trabecular bone is more abundant in the maxilla. Previous reports [Slaets et al. 2006, 2007] compared the healing process in these two bone types. It became obvious that trabecular bone heals more rapidly than cortical bone. However, cortical bone provides a firm mechanical interlocking of the implant immediately after installation [primary stability], which is also important when discussing the process of osseointegration. These differences in healing pattern should be taken into account when osseointegration of implants is anticipated in patients.

Secondly, this experiment was performed in rabbits. Although the duration of the remodeling cycle in the rabbit is 6 weeks, this cycle takes about 4 months in humans, indicating that a factor of 3 should be applied when extrapolating our results to the clinical situation. This suggests that in humans, bone remodeling is still ongoing 4 months after implant placement. Clinical research, however, remains the ultimate test to validate the former results.

Conclusion
These results show that the immediate loading protocol that was administered to the implants caused no large differences in the sequence of biological events leading to osseointegration in cortical bone, as there was the formation of a hematoma and an altered nuclear morphology of osteocytes surrounding the implantation site, followed by an intensive bone remodeling and the formation of new bone leading to the osseointegration of the implant. However, different rates in the new bone formation process were observed, mainly an elongation of the endosteal and periosteal bone formation process in case of immediate loading, as well as a reduction of the new bone formed at the cortical level.

Acknowledgements: This study was supported by the Research Council of the Catholic University of Leuven (OT/02/50) and by the Fund for Scientific Research Flanders (G.0162.03). J. Duyck is a postdoctoral Research Fellow of the Fund for Scientific Research Flanders. The authors wish to thank S. Jaecques and J. Vander Sloten for the use of the mechanical loading device. E. De Smet is gratefully acknowledged for her help and expertise regarding the implantation procedure. The authors also wish to express their gratitude to K. Sasaguri for his kind gift of the cbfa1 antibody and to AstraTech, Sweden, for providing the implants.

References


