Direct High-Frequency Stimulation of Peri-Implant Rabbit Bone: A Pilot Study

Xiaolei Zhang, DDS, PhD; Ignace Naert, DDS, PhD; Dorien Van Schoonhoven, DDS; Joke Duyck, DDS, PhD

ABSTRACT

Objective: This study aimed to evaluate the effect of direct high-frequency mechanical stimulation on the peri-implant tissue healing.

Materials and Methods: A total of 48 custom-made 2-mm diameter titanium implants were inserted in the tibial epiphyses of 12 rabbits. Half of the implants were stimulated by direct vibration (60 ± 10 Hz) immediately after insertion for 1 and 4 weeks, respectively. The other half served as controls. The samples were collected after the animals were sacrificed and were histologically processed into paraffin sections and stained with haematoxylin and eosin. The bone fraction was measured in an area of 50 and 400 μm around the implant. To rate significant differences a one-way analysis of variance was used with α set at 5%.

Results: No significant difference in bone fraction was found between test and control groups. When the bone fractions of the 50 and 400 μm peri-implant regions were compared, a significantly larger bone fraction was found in the 50 μm peri-implant region for the 4-week stimulated group.

Conclusion: Histomorphometric analyses could not reveal a pronounced effect of direct immediate high-frequency implant loading.

KEY WORDS: high-frequency, implant, loading, rabbit

INTRODUCTION

Bone is a metabolically active tissue capable of adapting its mass, shape, and structure to mechanical stimuli and repairing structural damage through the process of remodeling. The adaptation of bone in response to mechanical loading is considered to be a life-long process.[1] It is clear that dynamic loading induces bone formation other than static loading.[2,3] Different in vivo studies support the notion that bone is sensitive to the applied strain rate.[4–7] Over the past decade, by use of different animal models, the high-frequency low-magnitude stimulation, also referred to as vibration, has been proven to actively stimulate osteogenesis and improve the skeleton quality.[8] Most of the studies used an oscillating plate to induce whole body vibration as a stimulus.

For a long time it was assumed that mechanical loading during implant healing compromised peri-implant osteogenesis because of fibrous tissue formation, thereby impairing the osseointegration.[9,10] Micromotion at the implant–bone interface in case of immediate implant loading can lead to inhibition of bone formation at the interface because it probably interferes with the development of adequate early scaffolding of the fibrin clot. This event might disrupt the reestablishment of a new vasculature to the healing tissue, which in turn interferes with the arrival of regenerative cells. Eventually, the healing process is rerouted into repair by collagenous scar tissue instead of bone regeneration.[11,12] Ossification of regenerating tissue is only possible in case of a low hydrostatic strain environment and in the absence of shear strain.[13] Whereas, high-peak strains impair bone mineral formation and

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osteogenic cell differentiation, physiologic bone loading (500–3,000 microstrains) as such does not inhibit bone formation.[14] Premature implant loading can promote early peri-implant osteogenesis[14–18] in case of limited interfacial micromotion and an optimal biomechanical coupling between the implant and the surrounding tissues. Such an optimal biomechanical coupling implies the transfer of compressive or tensile rather than shear forces. Indeed, such loads on healing bone might even shorten the healing period.[19,20] As healing bone has lower mechanical properties compared with mature bone, equal loading will result in higher strains in immature bone. The load bearing capacity of healing bone is therefore less than mature mineralized bone. Both animal experiments[21,22] and clinical studies[12,23–25] have shown that immediately loaded oral implants acting as support for a prosthesis can osseointegrate provided that the forces and implant micro-motion are controlled.[20,26,27]

Nowadays, there is a tendency to load implants immediately or soon after implantation. This limits the period of discomfort for the patients compared with the long healing times in the delayed loading protocol. As the treatment goes faster, it also offers psychosocial and economic benefits. The local mechanical loading situation is believed to be a strong determinant in the processes of tissue differentiation and bone formation/resorption around implants. Early/immediate loading might offer the potential to stimulate osteogenic effects during implant healing under specific conditions.

Considering the abundant evidence of the positive effect of high-frequency loading on both bone adaptation and regeneration in general,[28] the idea of accelerating the osseointegration process and optimizing oral implant success through a therapeutically mechanical stimulation is tempting but not scientifically founded. This study therefore aims to evaluate the effect of high-frequency mechanical stimulation on the peri-implant tissue healing in the context of the optimization and acceleration of the osseointegration process.

MATERIALS AND METHODS

Implants and Animals

Custom-made 2-mm diameter commercially pure titanium (grade 2, Goodfellow, Huntingdon, United Kingdom) implants were used (see Figure 1). The screw

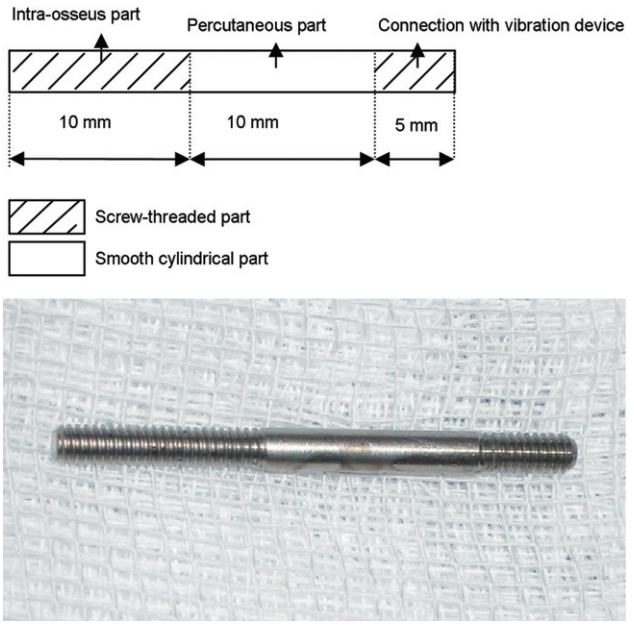

Figure 1 Design of the implant.

design of the upper part allowed a solid connection with the vibration device (Figure 2).

Prior to surgery, the implants were cleaned ultrasonically, decontaminated by soaking in a 4% hydroxyl-fluoride (HF) buffered with a 20% HNO_3 solution for 30 seconds, and sterilized. By doing so, the implant surfaces were standardized into an average roughness (Sdr, %) of 1, which was determined by a scanning white-light interferometer (Wyko NT 3300; Veeco Metrology Inc., Tucson, AZ, USA). Twelve 6-month old female New Zealand white rabbits (body weight, 3.7 kg ± 0.5 kg) were selected for this experiment. The rabbits were all

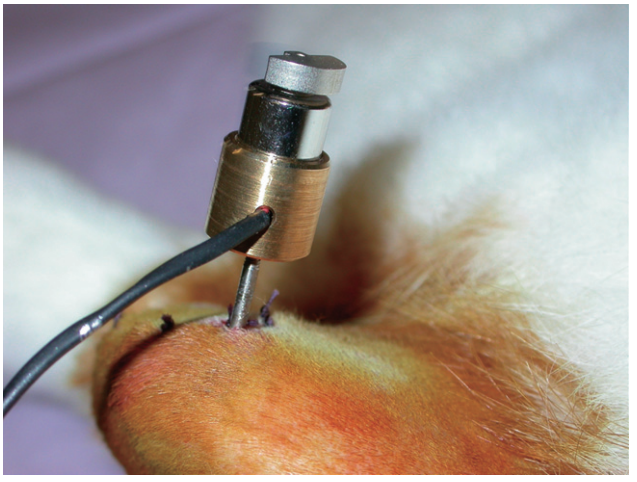

Figure 2 The vibration device was mounted on the implant.

pathogen free and were kept in quarantine for $1^1/_2$ months before the study was started. The study protocol was approved by the Regional Ethics Committee for Laboratory Animal Research of the Catholic University of Leuven and was performed according to the Belgian animal welfare regulations and guidelines.

Surgery and Loading Protocol

Under general anesthesia (intravenous, Diprivan 1%, 0.4 mL/kg h.; Astra Zeneca, Brussels, Belgium), a total of four implants were inserted in the two tibia epiphyses of each rabbit by two implant surgeries at 1 and 4 weeks before the sacrifice. During the first surgery, one implant was placed per leg. One of both implants received vibration stimulation; whereas, the other implant served as the unloaded control. After 3 weeks, a second implant was placed per leg. Again, one implant was loaded whereas the other one was not. After another week of healing, the animals were sacrificed with a 0.1 mL/kg intravenous injection of an embutramide, mebenzoniumiodide, and tetracaine hydrochloride solution (T61; Intervet, Mechelen, Belgium). By following this protocol, each rabbit contained a loaded and an unloaded implant that healed for 1 week and another loaded and unloaded implant that healed for 4 weeks. Postoperatively, the animals were given a dose of an intramuscular injection of 0.05 mg/kg buprenorphine as analgesics and 300.000E/d antibiotics (penicillin, Kela, Hoogstraten, Belgium) for 3 days.

A custom-made vibration device was used to apply the vibration (60 ± 10 Hz). Immediately after the implantation, the implants of the test group received vibration for 10 min/day, 5 days/week. The control implants did not receive any vibration stimulation, but underwent the sham manipulations (mounting of the vibration device) (Figure 2).

Tissue Processing and Histomorphometry

Immediately after animal sacrifice, the samples were harvested. The tissues were fixed in 4% paraformaldehyde for 3 days. The samples were immersed in 0.5 M EDTA (pH 7.4) and formic acid (HCOOH – pH 3.7) at 4C until the decalcification was achieved. After demineralization, specimens were dehydrated through an ascending ethanol series prior to paraffin embedding. After paraffin embedding and freezing (–30C), the implants were gently removed, after which the samples were reembedded. The most coronal part of the sample was cut into 5-µm thick sections (perpendicular to the implant axis) and stained with hematoxylin and eosin. The bone fraction in a region of interest (ROI) of 50 and 400 µm around the implant was evaluated by a semiautomatic system (Axiovision, Carl Zeiss Microimaging GmbH, Standort Göttingen, Germany) connected to a Zeiss light microscope (Figure 3). To eliminate the endochondral ossification which is mostly involved in long bone growth, the cartilage in the target region was excluded from the analysis (ie, the mineral bone fraction = summation of all mineralized tissues in the ROI/(area of the ROI – area of cartilage in the ROI)).

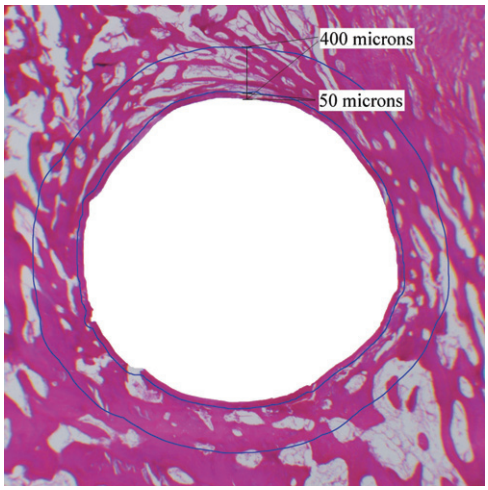

Figure 3 Illustration of the 50- and 400-µm range of interest in which bone fraction measurements were performed. The white space in the center represents the cavity where the implant was removed.

Statistics

Statistics were computed using a commercially available software package (SPSS, Chicago, IL, USA). The data were analyzed using one-way analysis of variance; *p*-values less than 0.05 were considered significant. The data were presented as the mean ± standard deviation (SD).

RESULTS

Means (SD) of the bone fraction in the 50 and 400 µm peri-implant areas are displayed in Figure 4. Neither in the 50 nor in the 400 µm peri-implant areas was a significant difference found between test and control groups. After a 4-week loading period, however, a significantly higher bone fraction was observed in the 50-µm compared with the 400-µm peri-implant region

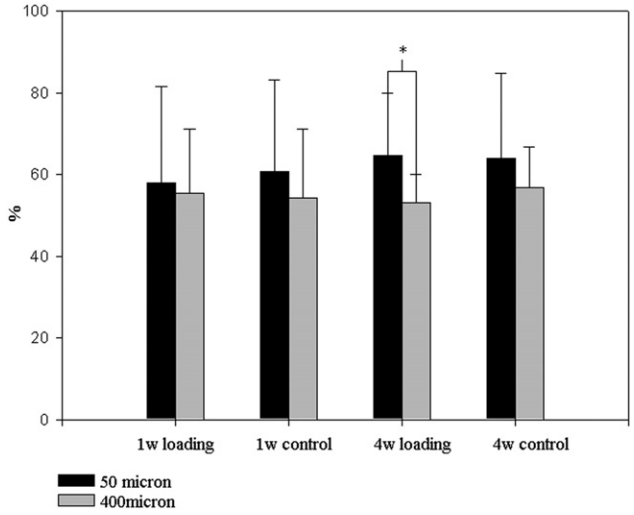

Figure 4 Peri-implant bone fraction in the 50-µm and 400-µm peri-implant areas (*$p < .05$).

(Figure 4). This difference in bone fraction depending on the distance from the implant was not observed around the unloaded implants.

DISCUSSION

In this study, the changes of the bone fraction in the peri-implant area in response to high-frequency direct implant loading were investigated. No significant differences in bone fraction were found between the loaded and the control group.

For both loading conditions and both healing periods, there was a tendency for a higher bone fraction in the immediate vicinity of the implant (50-µm peri-implant region) compared with the broader (400-µm) peri-implant region. The fact that this tendency was seen around both unloaded as loaded implants, in combination with the fact that the loading conditions did not significantly affect the peri-implant bone fraction, suggests that the effect of implant installation on the bone in the immediate vicinity of the implants might have overruled a positive effect because of the mechanical stimulation. Only after 4 weeks of mechanical stimulation, a significantly higher bone fraction was found close to the implant compared with in a broader zone around the implant.

This bone densification near the implant was also described by Balatsouka et al.[29] who found a significant increase in bone density from 2 to 4 weeks. The bone density of newly formed mineralized bone in the bone bed sites without implants, however, did not demonstrate a difference over time.

In this study, the bone fraction – as a representation of the bone density – was measured as this is the parameter that is commonly used for the evaluation of the effect of bone stimuli. Also, the bone-to-implant contact is often used as a parameter to quantify the implant osseointegration. This could not be done in this study because the implants were removed from the bone samples. The choice for paraffin sectioning required this removal of the implants. Previously, the effect of such implant removal on the surrounding tissues was investigated by means of scanning electron microscopy.[30] Implants removed only 1 to 3 days after insertion, showed scattered blood cells in a thin layer of (fibrin) matrix, which covered up to 30% of the implant surface. On implants removed after 1, 2, 4, or 6 weeks, some small sponge-like structures were rarely detected, which were devoid of cells. These structures were considered to be fibrous tissue or remnants of cells. The remaining surface of the implants was comparable with the surface of a blank implant. This means that, despite the removal of an implant from 4 weeks healed bone, the peri-implant tissues can be considered as relatively intact.

Because of both internal mediators and external mechanical demands, the bone tissue dynamically responds to a number of metabolic, physical and endocrine stimuli, undergoing continual chemical exchange and structural remodeling. Recent research has suggested that bones display an extraordinary adaptive behavior toward high-frequency low-magnitude mechanical stimulation.[28]

An early mechanical stimulation (implants healed for 1 week prior to stimulation) via indirect whole body vibration was applied on ovariectomized rats for 2 weeks at 50 Hz.[31] The peri-implant bone response was quantified by micro-computed tomography and revealed an increased amount of relative bone volume in a 48 µm peri-implant area.

The current study, however, failed to demonstrate such an osteogenic effect of direct high-frequency loading. Besides, this effect might have been overruled by the healing response, another reason might be that because of the high-frequency loading directly applied onto the implant, a considerably interfacial micro motion between implant and the surrounding tissues might have been evoked. It has previously been shown and hence, this is generally accepted that large micromotions on the implant–bone interface lead to soft

tissue encapsulation (fibroplasia) and therefore compromise osseointegration.[11,12]

A third possible reason why the mechanical loading did not enhance bone formation might be an inefficient load transfer from the implant toward the surrounding tissues. It is known that peri-implant osteogenesis requires a limitation of the interfacial micromotion as well as an optimal force transfer.[19,20] The surface of the used implants (Sdr: 1%) might have been too smooth to establish an efficient biomechanical coupling during the initial healing period. Because of this lack of biomechanical coupling combined with the interfacial micromotion at the start of the mechanical stimulation, the mechanical stimulation failed to enhance bone formation.[32]

In silico modeling might contribute to a better understanding of the biomechanical conditions of this experimental set-up. However, the roughness of the implants (Sdr: 1%) was purposely kept low so as to not interfere with the pure mechanical bone response, which was the aim of the study. As well established, surface roughening speeds up the healing process in the first weeks after implant installation compared with turned implants.[20,33]

Further, to enhance osteogenesis around the implant, the strategy of biologic and/or geometric surface modification was explored in different animal studies. Coating with bioactive molecules on the implant surface (for instance, collagen and chondroitin sulphate, bone morphogenetic protein-2, calcium phosphate, bisphosphonate) might hold the potential to stimulate bone-to-implant contact[34,35] or does increase removal torque values.[36] Nevertheless, in all the pervious mentioned studies, the relative peri-implant bone density was not found to be different, which is consistent with our results. It is likely, that in case of biologic and/or local mechanical stimulation, the osteogenic effect is more predominant in the vicinity of the bone–implant interface rather than at distance. This osteogenic effect is not necessarily translated into an increased peri-implant bone fraction, as explained above, but rather, in an increased bone-to-implant contact.

As mentioned previously, several oscillation studies[37–41] indicated that very small load magnitudes (inducing 5 microstrains or less) may already have osteogenic potential when the stimulation is applied at high frequency (>30 Hz). Despite a similar loading regime used in this study, a convincing osteogenic effect could not be observed when the stimulation was directly evoked through an implant. A positive outcome might have been more likely though when the high-frequency loading was applied on the surrounding bone rather than directly onto the implant. This would avoid implant micro-motion, but could still stimulate the peri-implant tissues, which in turn might have a positive effect on peri-implant bone healing and eventually osseointegration. This is subject in ongoing investigation.

In conclusion, no pronounced effect of immediate high-frequency loading, which was applied at 60 ± 10 Hz with the direct vibration set-up in this study, could be observed on the peri-implant bone response. The bone healing processes appeared to have a more important effect compared with the mechanical loading.

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