THE EFFECT OF STIMULATION AMPLITUDE AND FREQUENCY ON PERI-IMPLANT BONE ADAPTATION IN THE GUINEA PIG MODEL

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Abstract

The presented work is part of a larger project to analyse adaptive bone response around implants receiving controlled mechanical stimulation early post-operatively. A sinusoidally varying mechanical stimulus can be characterised by the strain rate amplitude (SRA=strain amplitude·frequency). The hypothesis that strain amplitude and frequency cannot be considered independent stimulation parameters, was studied in a guinea pig model. For three series of guinea pigs, stimulation force amplitude and frequency were varied, so as to result in the same SRA. The number of cycles was adjusted together with frequency to keep stimulation time constant. Stimulation was applied daily during four weeks. Micro-focus computed tomography (CT) was used for *in vivo* follow-up and post-mortem evaluation. Bone adaptive response was quantified morphometrically. Data were processed statistically with SAS® 9.1. Overall, the largest increase in bone mass was observed in the first two weeks of stimulation. In the final two weeks of stimulation there was no significant change of bone mass. The effect of early mechanical stimulation of peri-implant bone is strongly dependent on force amplitude and frequency and not on strain rate amplitude. The guinea pig cortical bone model is most sensitive for high amplitude/low frequency stimulation.

Introduction

The local mechanical loading situation is believed to be strongly determinant in the processes of tissue differentiation and bone formation and resorption around implants [1-3]. This study was part of a larger project with the ultimate objective to develop a model of tissue differentiation that may predict the *in vivo* bone response on specific mechanical loading parameters. With such a model, mechanical stimulation parameters that accelerate or consolidate osseo-integration in cases of early and immediate loading can be selected, or the design of the implants can be optimised in function of early and immediate loading.

A sinusoidally varying mechanical stimulus can be characterised by the strain rate amplitude (SRA = strain amplitude · frequency). Important parameters for osteogenic (bone forming) mechanical stimulation can be identified from literature (e.g. SRA, peak strain, number of cycles or duration) [4]. However, the relationships among these parameters and peri-implant bone response are not yet fully understood.

From previous work, an optimally osteogenic SRA of 1600 $\mu\varepsilon.s^{-1}$ was estimated for a percutaneous implant in a guinea pig model [5]. Literature suggests that strain amplitude and loading frequency cannot be considered independent stimulation parameters [3]; this study examines this hypothesis in that guinea pig model. Stimulation regimes with various combinations of applied force amplitude and frequency were applied, resulting in the same strain rate amplitude on the peri-implant bone. Micro-focus CT was used for the *in vivo* follow-up of peri-implant bone(re)modelling [5,6].

Materials and methods

Three series of male, skeletally mature guinea pigs (average 11 per series) received percutaneous customised implants (Astra Tech, Mölndal, Sweden) in the distal part of both tibiae. The screw-shaped Ti6Al4V implants were bicortically fixated. In each animal, one implant was stimulated one week after implant installation while the contra-lateral implant served as unloaded control. A sinusoidally varying bending moment was applied with a force-controlled electro–mechanical shaker. All series received a stimulation of 10 min/day, 5 days/week, during 4 weeks. The strain amplitudes listed in table 1 were calculated from cadaver strain gauge measurements, in the direction of stimulation, at 2.3 mm from the implant centre. Varying force amplitudes were combined with varying frequencies of stimulation to result in a SRA of 1600 $\mu\varepsilon.s^{-1}$. The number of cycles was also adjusted so the stimulation time was 10 minutes for all three series.

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Table 1: Mechanical parameters of the early stimulation experiment of screw-shaped implants in the distal tibia of guinea pigs.

Series code	Cycle number (-)	Force amplitude (N)	Strain amplitude (με)	Frequency (Hz)	Strain rate amplitude ($\mu\varepsilon.s^{-1}$)
Ib	1800	2	533	3	1600
Ic	6000	0.6	160	10	1600
Id	18000	0.2	53	30	1600

The micro-CT device is a HOMX 161 microfocus X-ray source (Philips X-ray, Hamburg, Germany) equipped with an AEA Tomohawk rotating sample stage and image acquisition and slice reconstruction software. *In vivo* μCT scans both of test- and control sides were taken post-operatively before stimulation was started (v1), after two weeks of stimulation (v2) and at the end of the study after four weeks of stimulation (v3). Limited radiation exposure was considered. The animals were sacrificed by asphyxiation with carbon dioxed gas, tibiae were dissected and *post mortem* micro-CT scans (*pm*), using different scan parameters resulting in a higher resolution, were taken of the bone-with-implant specimens. The micro-CT protocol is detailed in Jaecques *et al.* (2004) [6]. Micro-CT slices were reconstructed also in the loading direction parallel to the longitudinal direction of the tibia and to the long axis of the implants. A micro-CT slice through the middle of the implant was selected for each time point (*v1, v2, v3, pm*). The effect of stimulation on bone(re)modelling was quantified morphometrically (Q-win® image analysis software, Leica BV, Rijswijk, The Netherlands) by comparing bone mass around control and stimulated implants. Bone mass was defined on micro-CT slices as the bone-occupied area fraction of a standardized region of interest (ROI). Bone mass (BM) (%) = (area of bone in the ROI (μm^2) / ROI area (μm^2)) * 100. ΔBM = BM at the stimulated side minus BM at the control side. ROIs (Figure 1) were defined in the cervical peri-implant cortex (C), marrow cavity (M) and apical cortex (A) at 500, 1000 and 1500 μm from the implant surface, at the distal and proximal side of the implant; these ROIs are referred to as e.g. M500, M1000, etc. The protocols for all guinea pig experiments were approved by the ethical committee of the animal research facility of the K.U.Leuven University.

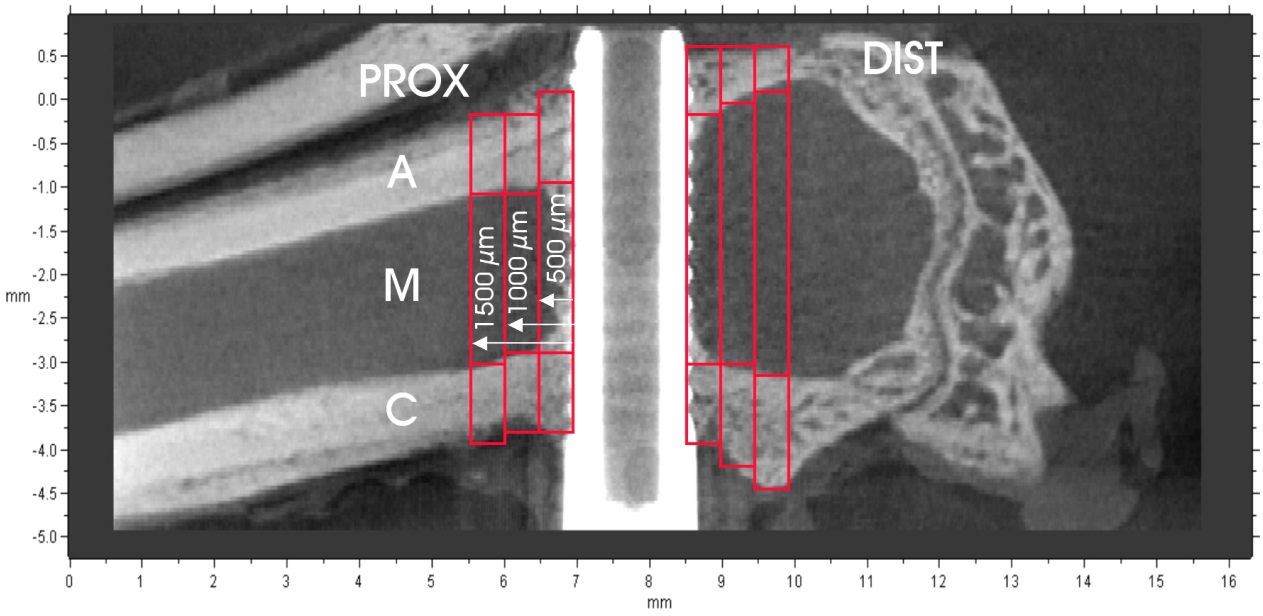

Figure 1: example of a post-mortem μCT-slice of one guinea pig tibia with percutaneous implant with indicated ROIs for bone mass measurements.

Results and discussion

Typical results of 2D reconstructed CT-slices clearly showed progressive bone remodelling over four weeks (Fig 2). On the *in vivo* image, somewhat more streak artefacts and general noise could be seen. However, the geometry of the cortical bone on the *in vivo* image matched the *post mortem* image quite well. The results were also usable as input for the generation of individualised finite element models; this is reported elsewhere [6].

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In the medullary cavity, where there was no bone in contact with the implant at the start, bone (re)modelling was the most obvious and the effect of mechanical stimulation can be shown the clearest. On the longitudinal slices it can be clearly shown that for the medullary cavity new bone is formed along the surface of the implant. This bone originates from the endosteum of the cervical and apical cortical bone and covers by osteoconduction the surface of the implant. Not only along the implant surface but also along the endosteum away from the implant, new bone is formed in the medullary cavity. The area of new bone formed from the cervical endosteum is systematically wider than along the apical endosteum. Systematically more remodelling activity can be seen in the cervical cortex than in the apical cortex, both for test and control implants.

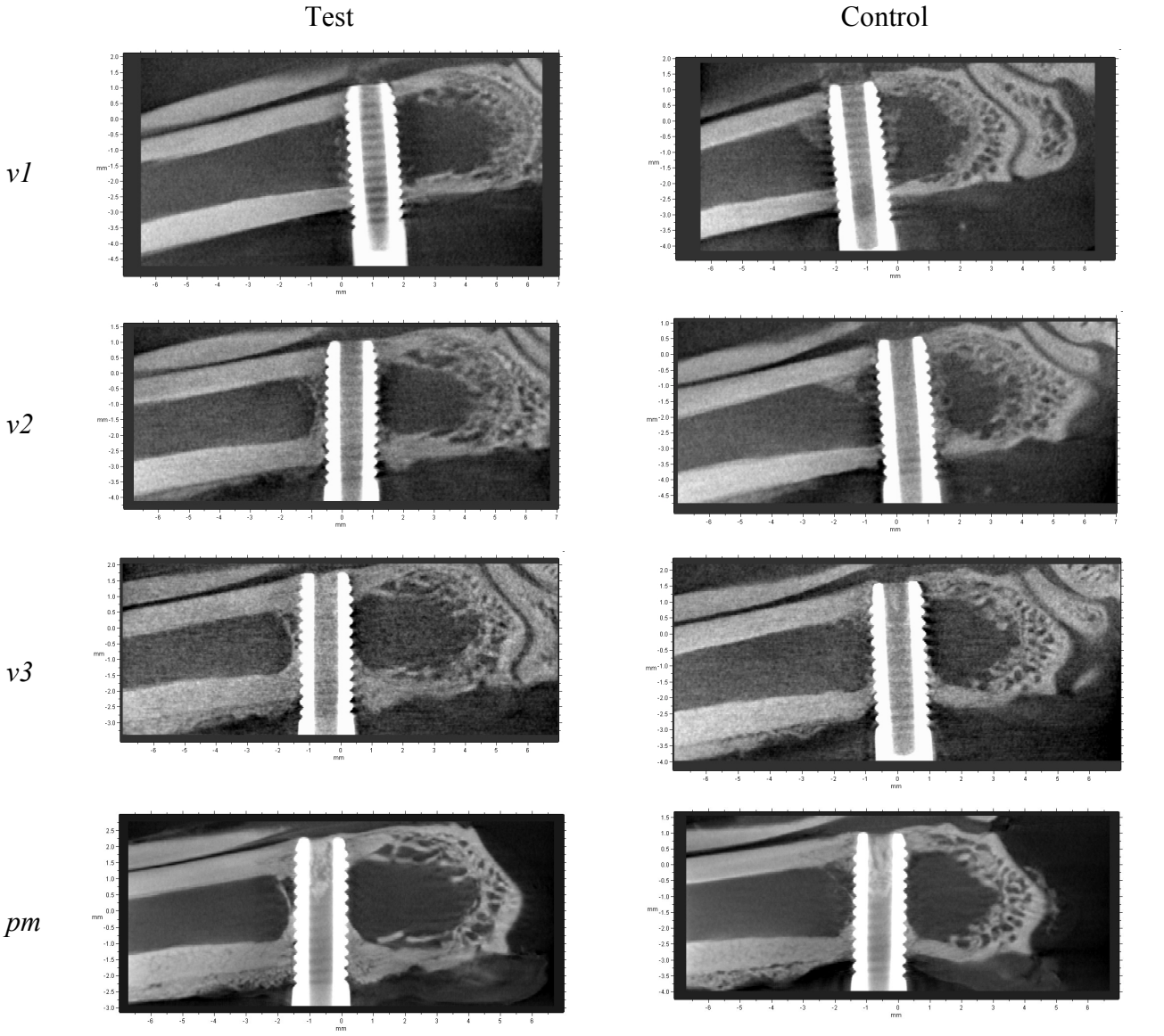

Figure 2: Example of 2D reconstructed CT-slices of test and control tibia of a guinea pig post-operatively before stimulation (v1), after 2 weeks of stimulation (v2), after 4 weeks of stimulation (v3) and post mortem at that same time point (pm).

To obtain valid inferences about the mean evolution of bone mass and its relationship with the variables of interest, the correlation between measurements on the same guinea pig (control/test and across time) should be accounted for. A multivariate linear model in SAS® 9.1 to study the joint evolution of bone mass around the test and control implant over time was fitted. The model included stimulation (i.e. test/control), time period (i.e. *v1/v2/v3*) and their two-way interaction terms as fixed effects with an appropriate variance-covariance matrix

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in each region separately. To study the effect of series on the evolution of bone mass, a multivariate model was also fitted, including series, stimulation, time period and their two-way interaction terms with an appropriate variance-covariance matrix. The *post mortem* data were analysed by fitting a linear mixed model with an appropriate variance-covariance matrix to account for the correlation between measurements on the same guinea pig (control/test). The model included series, stimulation and their interaction as fixed effects. To adjust for multiple testing, the Tukey procedure was used. The level of significance was set at 5%.

The evolution of bone mass, pooled for test and control implants, is shown in figure 3. Considering the M-500 region, with respect to time period, the significant differences are between v2 and v1 ($p<0.0001$) and between v3 and v1 ($p<0.0001$), but not between v2 en v3 ($p=0.018$). This means that bone(re)modelling especially occurred during the first two weeks of stimulation. Already during these first two weeks, meaning at timepoint v2, there is a significant effect of series ($p=0.004$) and of stimulation ($p=0.0113$). For the regions M1000 and M1500, an increase in mean bone mass can also be shown, but the effect decreases with increasing distance to the implant.

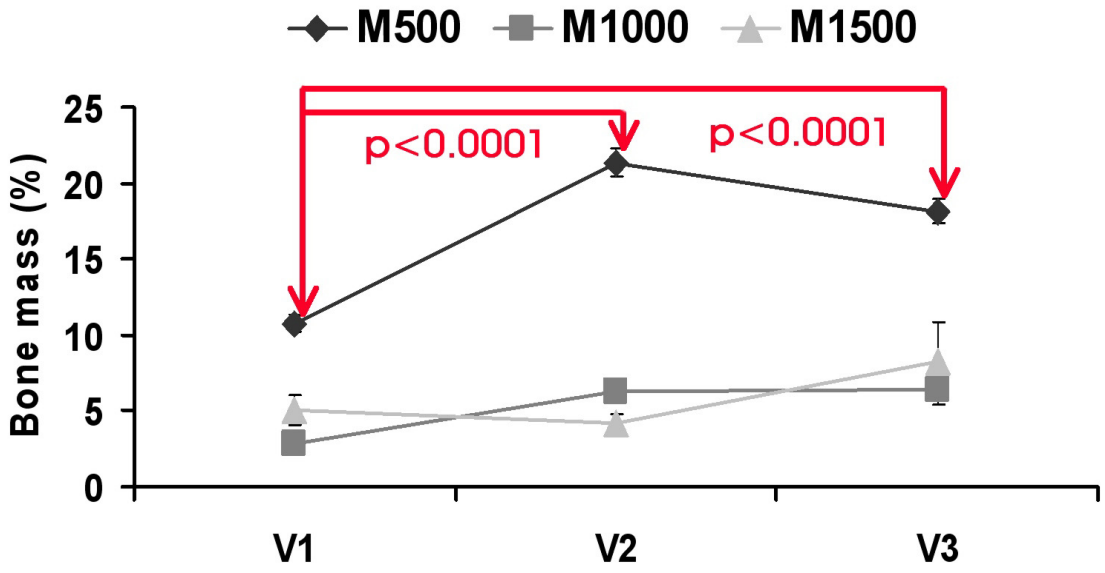

Figure 3: average bone mass in the marrow cavity, pooled for test and control implants and for all three series, at time points v1 (one week after implantation), v2 (after two weeks of stimulation) and v3 (after 4 weeks of stimulation). Symbols indicate the distance to the implant (500, 1000 and 1500 µm respectively). The size of the symbols obscures some of the error bars.

Figure 4 shows Δbone mass between test and control sides for the M-regions after four weeks of stimulation, measured on the *pm* micro-CT slices. There is a statistically significant difference in the effect of early mechanical stimulation between series Ib versus Ic ($p=0.006$) and Id ($p=0.016$) for the M500-region.

In this model, although the same strain rate amplitude was applied, the effect of early mechanical stimulation decreased with decreasing force amplitude and increasing frequency.

This confirms earlier preliminary observations [5] where high frequency/low amplitude stimulation, although characterised by a higher SRA, had less effect on Δbone mass than low frequency/high amplitude stimulation with a lower SRA.

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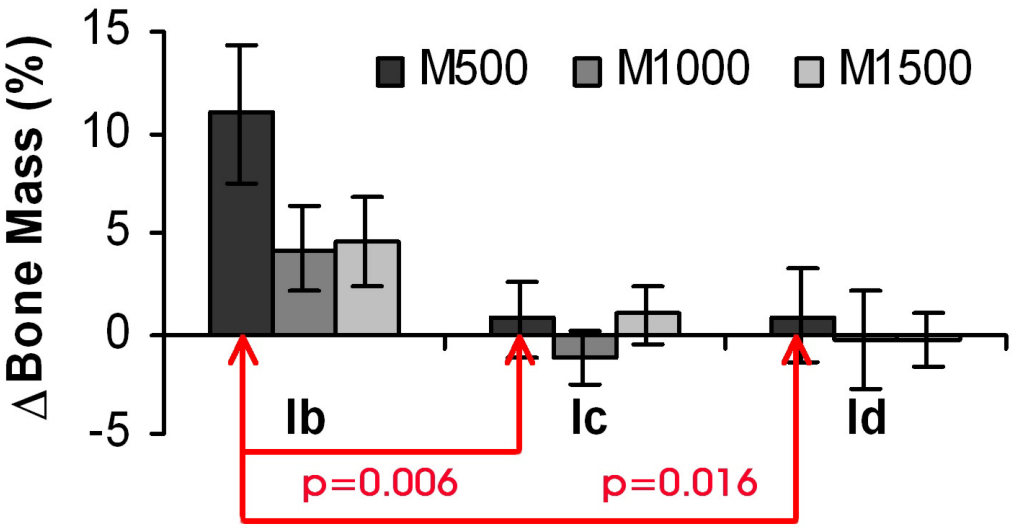

Figure 4: average Δbone mass (%) of proximal and distal side of the implant for series Ib, Ic and Id for regions M500, M1000 and M1500 at the end of the experiment, measured on post mortem μCT slices.

Conclusion

The effect of early mechanical stimulation of peri-implant bone is strongly dependent on force amplitude and frequency and not on strain rate amplitude. This cortical bone model is most sensitive for high amplitude/low frequency stimulation.

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