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PICCININI, Marco, et al.

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Reference

DOI : 10.1111/clr.12760
PMID : 26864329
Peri-implant bone adaptations to overloading in rat tibiae: experimental investigations and numerical predictions

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Key words: adaptation algorithm, bone augmentation, finite element, implant integration, in vivo stimulation, specimen-specific

Abstract
Objectives: (i) To assess the effects of mechanical overloading on implant integration in rat tibiae, and (ii) to numerically predict peri-implant bone adaptation.

Materials and methods: Transcutaneous titanium implants were simultaneously placed into both tibiae of rats (n = 40). After 2 weeks of integration, the implants of the right tibiae were stimulated daily for 4 weeks with loads up to 5N (corresponding to peak equivalent strains of $3300 \pm 500 \mu \text{strain}$). The effects of stimulation were assessed by ex vivo mechanical tests and quantification of bone mineral density (BMD) in selected regions of interests (ROIs). Specimen-specific finite element models were generated and processed through an iterative algorithm to mimic bone adaptation.

Results: Bilateral implantation provoked an unstable integration that worsened when mild (2–4N) external loads were applied. In contrast, a stimulation at 5N tended to “counterbalance” the harmful effects of daily activity and, if applied to well-integrated specimens, significantly augmented the implants’ resistance to failure (force: +73% $P < 0.01$, displacement: +50% $P < 0.01$ and energy: +153% $P < 0.01$). Specimen-specific numerical predictions were in close agreement with the experimental findings. Both local and overall BMD variations, as well as the implants’ lateral stability, were predicted with small errors (0.14 gHA/cm$^3$ and 0.64%, respectively).

Conclusions: The rats’ daily activity detrimentally affects implant integration. Conversely, external stimulations of large magnitudes counterbalance this effect and definitively improve integration. These changes can be predicted using the proposed numerical approach.

Metal implants are typically used as endosseous anchorage to replace missing teeth. When fitted with a restoration, they assist in restoring oral function (mastication and speech) and the patients’ normal appearance.

The typical dental implant is cementless. It is the interaction between the implant surface and the surrounding bone that is critical in promoting osseointegration, that is, a direct apposition of the osseous tissue onto the metal. The adhesive strength of the bone-metal interface, though, is actually quite low (Gross et al. 1987; Takasuka et al. 1995), and it is the implants’ surface texture that permits a mechanical gripping of the bone onto the implant (Cochran et al. 1996). After initial osseointegration, the stimulation of the supporting bone by the loaded implant maintains bone volume while “understimulated” bone will resorb (Huiskes et al. 1992).

The interplay between implant and bone thus ensures the homeostasis of the bone bed. As such, it has been the object of a number of investigations (Goodacre et al. 1999; Albrektsson 2008). In an effort to refine the analysis, implants were inserted into the tibiae of dogs (Hoshaw et al. 1990), rabbits (Duyck et al. 2001; Han et al. 2014), or guinea pigs (De Smet et al. 2005). The implant heads protruding from the skin were loaded, with the aim of transferring controlled stresses to the supporting bone. Axial- (Leucht et al. 2007), torsional- (Van Der Meulen et al. 2006), or bending force systems (De Smet et al. 2006) have been implemented. In such models, the implant-mediated mechanical stimulation of the supporting bone induces bone formation or resorption depending on the load levels applied. These phenomena in turn affect the bone’s strength (Lee et al. 2006).
implant integration. In this context, our group developed the “loaded implant” model in which two transcutaneous cylindrical implants are inserted mono- or bi-cortically into the tibiae of rats. A computer-driven actuator forces the implant’s heads together thus generating controlled stress fields within the bone bed (De Smet et al. 2005; Wiskott et al. 2008, 2012). Previous test campaigns using this animal model clarified the relationship between external implant loading and intra-osseous strains [Piccinini et al. 2012]. For instance, a mild external stimulation improved integration and resulted in an increased resistance of the implants to pullout (Zacchetti et al. 2013). Conversely, in some animals, the mere stresses arising during the animal’s normal daily activity generated tensile strains (~1200 microstrain between implants) and stresses in the cortical bone of magnitudes such that the bond between the implant and the surrounding tissue was severed. The ensuing cortical bone recession largely contributed to the complete loss of bone–implant integration as observed in several instances (Piccinini et al. 2014).

In this study, the “loaded implant” model was used to investigate a “critical” [i.e. high stress] mechanical environment. Both tibiae were implanted simultaneously. Hence, the effects of daily activity were fully transferred to the bones by preventing the animal from sparing [i.e. unloading] either leg. In addition, the implants were externally loaded to 5N, 5 days per week for 4 weeks, thus subjecting the bone–implant interface to additional levels of compressive strain in the 3300 ± 500 με range. The resulting data were used to generate specimen-specific finite element [FE] models of implanted rat tibiae by implementing the procedure described by Piccinini et al. [Piccinini et al. 2012]. The models were processed using an iterative algorithm with the aim of generating numerical predictions of bone adaptation and implant integration.

Material and methods

In vivo stimulation

The experiment was conducted on 40 female Sprague Dawley rats. The animals were 27 weeks old at the onset of the experiment. After initial acclimation, the animals were pair-fed for 2 weeks. Then, two titanium implants were surgically inserted into the proximal part of each tibia with their heads protruding from the skin. The distal implants were anchored bicortically while the proximal implants only penetrated the medulla by ca. 3 mm [Fig. 1a]. The technical aspects of the surgery, the implant design, and the activation setup that characterizes the “loaded implant” model have been described elsewhere (De Smet et al. 2005; Wiskott et al. 2012; Zacchetti et al. 2013). After surgery, the animals were caged under standard conditions, with free access to food and water for 2 weeks of post-surgery healing. At the end of this period, they were divided into five groups. One group was sacrificed at this time as representative of the initial integration state (i.e. the “basal group”). The remaining four groups underwent 4 weeks of external stimulation of the right tibiae at 2, 3, 4, and 5 N, respectively. These load levels corresponded to estimated internal strains of 1320 ± 200 με, 1980 ± 300 με, 2640 ± 400 με, and 3300 ± 500 με, respectively [Piccinini et al. 2012]. Activation consisted in cyclically forcing the implants heads together [Fig. 1a] under the following protocol: 1 Hz sinusoidal cycle, 900 cycles/day, 5 days/week, with a progressive increase of loading during the first week (1N/day, Fig. 1b). The left tibiae were not stimulated [i.e. the “unstimulated control”]. The animals were fitted with collars to prevent them from gnawing on the surgical sites. After sacrifice, all tibiae were dissected, cleared of their soft tissue coverage, and frozen to −21°C.

In line with Swiss regulations, the experiment was approved by the University of Geneva’s animal rights committee and supervised by the local veterinary agency.

Computed tomography analyses

Prior to computed tomography [CT] scanning, the specimens were thawed, maintained at 4°C for 24 hours in a 0.9% solution of NaCl, and then gradually brought to room temperature. The specimens were analyzed using a high-resolution CT imaging system (μCT-40, Scanco Medical AG, Brüttisellen, Switzerland).

![Fig. 1. (a) Implanted specimen. The proximal implant is inserted monocortically (i.e. “free-floating” inside the trabecular bone) while the distal implant is anchored bicortically. Arrows: direction of stimulation. (b) Time schedule of the experiment.](image-url)
Switzerland) with the following settings:

- 1022 slides × 360 degrees of rotation,
- isotropic voxel size: 20 μm, source potential: 70 kVp,
- tube current: 114 μA, integration time: 320 ms,
- beam hardening correction: 200 mgHA/cm³.

Local bone mineral density (BMD) was assessed from the scans through ITK-snap [Yushkevich et al. 2006] in six regions of interest (ROIs) as shown in Fig. 2.

**Ex vivo mechanical tests**

The specimens were loaded *ex vivo* to determine the implants’ lateral stability, that is, inter-implant strain, ultimate displacement, force, and energy. The tests were carried out using an electromechanical system (5848 Microtester, Instron Corp., High Wycombe, UK). The machine’s crosshead displacement was measured while applying a controlled load to the implant heads [Fig. 3]. The implant heads were aligned vertically through sharp, V-shaped notches, machined on two 1.5-mm-thick steel plates. Each specimen was subjected to five cycles ranging from 1 to 5N at a rate of 0.01 mm/min to determine the inter-implant strain (set as the crosshead displacement divided by the inter-implant heads’ distance at rest). The ultimate force \( F_U \) and displacement \( d_U \) were measured by loading the specimens to failure at 0.01 mm/min. The ultimate energy \( U_U \) was calculated as

\[
U_U = \int_0^{d_U} F_U(x) \, dx
\]  

The elasticity inherent to the experimental setup was evaluated in separate tests. The machine’s crosshead displacement was adjusted accordingly.

**Numerical analyses**

The experimental baseline was set by the basal group which comprised those animals that had undergone 2 weeks of integration only and thus characterized a “standard” integration with no stimulation.

Five individual FE models of implanted rat tibiae were generated from five “basal” specimens. The μCT scans were segmented and analyzed using the procedure described by Piccinini et al. [Piccinini et al. 2012]. In this procedure, the CT images are processed with an open-source FE model generator, VoxelMesher [Piccinini et al. 2012], to quality second-order tetrahedral meshes. Then, the nodes are assigned isotropic material properties according to their (CT-determined) BMD using the BMD–elasticity relationship developed by Cory et al. [Cory et al. 2010]. This technique treats bone as a continuous medium and is typically employed for studies involving whole bones [Terrier et al. 1997; Taddei et al. 2004]. Indeed, the trabecular reticulum is not resolved, but characterized by local mechanical properties that depend on tissue density.

The simulations of bone adaptation were phenomenological and based on Frost’s mechanostat [Frost 1987]. The mechanostat stipulates that bone adjusts the mechanical stimulus to which it is subjected to a reference range [i.e. the “lazy zone”] by reducing or increasing bone mass [Beaupré et al. 1990]. When dealing with implanted bones, volumetric adaptation [i.e. extra-cortical bone apposition] is often neglected, while “internal” adaptation is modeled by adapting the bone’s density in reaction to the mechanical stimulus [Terrier et al. 1997]. More specifically, our modeling strategy involved the iterative adjustment of BMD depending on the computed octahedral shear strain [octahedral shear strain was identified as the most effective stimulus for the implementation of the mechanostat theory at the continuum level [Piccinini 2014]].

The BMD variation was computed using the formulation of Eqn. 2 which was adapted from the quadratic form proposed by Li et al. [Li et al. 2007].

\[
\frac{d \rho}{dt} = \begin{cases} 
K_a(\psi - \psi_d) & \text{if } \psi \leq \psi_d \\
0 & \text{if } \psi > \psi_d 
\end{cases}
\]  

where \( \rho \) is the BMD, \( \psi \) is the octahedral shear strain, \( K_a \) is the adaptation rate, \( \psi_d \) and
ψ₀ are the damage and apposition adaptation thresholds. The BMD variation rate $dρ/dt$ is plotted vs. the mechanical stimulus in Fig. 4. In this formulation, there is (i) no effect of implant loading if the stimulus is low $|dρ/dt| < 0$ if $ψ < ψₐ$; (ii) a positive effect of loading if the stimulus ranges in the apposition domain $|dρ/dt| < 0$ if $ψ < ψₐ < ψₜ$; and (iii) bone resorption due to overloading when the stimulus exceeds the damage limit $|dρ/dt| > 0$ if $ψ < ψₜ$. Due to lack of pertinent data, disuse due to “understimulation” was not considered (Crupi et al. 2004; Li et al. 2007) as it was surmised that the animals’ daily activity prevented any “disuse.”

The computational procedure also included a spatial averaging of the stimulus over a spherical zone of influence (ZOI) (Mullender et al. 2004; Li et al. 2007) as it was surmised that the animals’ daily activity prevented any “disuse.”

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The mechanical environment implemented in the present experimental protocol produced conflicting outcomes as it disintegrated a number of implants and enhanced the integration of others. As the animals were not given the option to unload the implanted legs during healing, osseointegration was relatively poor and some animals hardly adapted to the conditions of the experiment. Implants were lost in all experimental groups independently of the load applied after the healing phase. Table 1 summarizes the observations. Five proximal implants were lost during healing, and 18 (11 right and 7 left) were lost during the stimulation phase. Most implants were lost during weeks 3–4 (i.e. the first and second weeks of stimulation). Mainly loaded implants were affected (Fig. 6a). Two right proximal implants integrated but rotated by 90 degrees with respect to their axis, thus hampering stimulation. In total, 15 rats were prematurely euthanized due to implant losses and infections (38% of total). Load level and implant loss were not linearly correlated, as shown in Fig. 6b. Mild stimulation levels (i.e. 2, 3 and 4N) deteriorated the state of integration more than did maximum loading (5N). Interestingly, the specimens that resisted the stresses imposed with the continuum assumption [3 to 5 inter-trabecular lengths according to Bouxsein et al. (Bouxsein et al. 2010)]. $σ$ was set to 0.4085 so that $f(Dₜ) = 1$ if $r = 0$ mm (at the central node) and $f(Dₜ) = 0.05$ if $r = 0.3$ mm (the ZOI limit). As only the steady state solution was of interest, the adaptation rate $Kₜ$ was assigned the value 1 [gHA/cm³/(time unit)]. The apposition and damage attractor states were $ψₐ = 1.25 \times 10^{-3}$ and $ψₜ = 4.5 \times 10^{-4}$ in agreement with previously published laws of strain-based adaptation (Frost 1990; McNamara & Prendergast 2007) and the physiological deformation of the rat tibia during gait (Piccinini et al. 2014).

Equation 2 was iteratively solved through forward Euler integration to adjust BMD as a function of bone deformation. Technically, the variation in BMD was calculated at each iteration using an adaptive time stepping. This algorithm optimized the time step in constraining the simulation process during the initial iterations, that is, whenever large variations of BMD occurred, but accelerated convergence when the error in bone adaptation was small (Van Rietbergen et al. 1993). The iterations stopped when a convergence criterion was satisfied. In the present instance, when 99.9% of nodes showed null adaptation errors.

To streamline the procedure, bone-to-implant contact was treated as perfectly adherent. Nonetheless, to prevent unrealistic load transfer to the bone whenever the interface was subjected to traction, a small modulus of elasticity was assigned to those regions as shown in Fig. 5.

Results

General clinical observations

The mechanical environment implemented in the present experimental protocol produced conflicting outcomes as it disintegrated a number of implants and enhanced the integration of others. As the animals were not given the option to unload the implanted legs during healing, osseointegration was relatively poor and some animals hardly adapted to the conditions of the experiment. Implants were lost in all experimental groups independently of the load applied after the healing phase. Table 1 summarizes the observations. Five proximal implants were lost during healing, and 18 (11 right and 7 left) were lost during the stimulation phase. Most implants were lost during weeks 3–4 (i.e. the first and second weeks of stimulation). Mainly loaded implants were affected (Fig. 6a). Two right proximal implants integrated but rotated by 90 degrees with respect to their axis, thus hampering stimulation. In total, 15 rats were prematurely euthanized due to implant losses and infections (38% of total). Load level and implant loss were not linearly correlated, as shown in Fig. 6b. Mild stimulation levels (i.e. 2, 3 and 4N) deteriorated the state of integration more than did maximum loading (5N). Interestingly, the specimens that resisted the stresses imposed with the continuum assumption [3 to 5 inter-trabecular lengths according to Bouxsein et al. (Bouxsein et al. 2010)]. $σ$ was set to 0.4085 so that $f(Dₜ) = 1$ if $r = 0$ mm (at the central node) and $f(Dₜ) = 0.05$ if $r = 0.3$ mm (the ZOI limit). As only the steady state solution was of interest, the adaptation rate $Kₜ$ was assigned the value 1 [gHA/cm³/(time unit)]. The apposition and damage attractor states were $ψₐ = 1.25 \times 10^{-3}$ and $ψₜ = 4.5 \times 10^{-4}$ in agreement with previously published laws of strain-based adaptation (Frost 1990; McNamara & Prendergast 2007) and the physiological deformation of the rat tibia during gait (Piccinini et al. 2014).

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To streamline the procedure, bone-to-implant contact was treated as perfectly adherent. Nonetheless, to prevent unrealistic load transfer to the bone whenever the interface was subjected to traction, a small modulus of elasticity was assigned to those regions as shown in Fig. 5.
by the animal’s daily activity also adapted well to stimulation at 5N, showing improved resistance to lateral force application.

**Effect of external stimulation on implant osseointegration: in vivo findings and numerical simulations**

For this study, the analysis was conducted using the load regimens considered as boundaries of the mechanical environment, that is, “basal,” “unstimulated control,” and “stimulated to 5N.”

The “stimulated” group comprised five specimens which resisted the mechanical conditions imposed [i.e. sites in which no cortical troughing occurred]. The “basal” group comprised the five remaining specimens after 2 weeks of integration while the “unstimulated control” group was formed by randomly selecting five control legs of groups 2,3, and 4 [i.e. the left legs of all animals were left unstimulated]. Hence, a total of 15 specimens were analyzed.

In all instances, either the right or the left leg of an animal was included into the analysis (never both). Therefore, the groups were “statistically independent.” A Mann-Whitney U-test with a significance level set to 0.05 was used to evaluate the differences between groups.

The specimens were selected for an extensive evaluation of their osseointegration and mechanical properties of specimens that resisted the mechanical environment, thereby providing the basis for numerical simulations and predictions. Table 2 lists the inter-implant mechanical strain variation as compared to that measured experimentally. The difference between specimens is substantial [i.e. minimum −7.0%, maximum −3.9%]. Still, the calculated inter-implant strain variation is in line with the average result of the experiments (+0.64%).

**Discussion**

In the present test campaign, we investigated the osseous tissue’s reaction to a critical mechanical environment by adding external

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**Table 1. Report of experiments**

<table>
<thead>
<tr>
<th>Group</th>
<th>Limb swelling</th>
<th>Implant loss</th>
<th>Integration period</th>
<th>Stimulation period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>L</td>
<td>Week 1</td>
<td>Week 2</td>
</tr>
<tr>
<td>Basal</td>
<td>2</td>
<td>1</td>
<td>–</td>
<td>1Lp</td>
</tr>
<tr>
<td>2N</td>
<td>3</td>
<td>3</td>
<td>1Lp</td>
<td>1Lp 1Rp</td>
</tr>
<tr>
<td>3N</td>
<td>4</td>
<td>4</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4N</td>
<td>3</td>
<td>2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5N</td>
<td>3</td>
<td>2</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

L: left tibia, R: Right tibia; p: proximal implant; d: distal implant; RX percentage of right tibiae (i.e. stimulated) prematurely lost.

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**Table 2. Results of the ex vivo mechanical tests**

<table>
<thead>
<tr>
<th>Ultimate properties</th>
<th>Group (n = 5)</th>
<th>Inter-implant strain ($)</th>
<th>Force (N)</th>
<th>Displacement (mm)</th>
<th>Energy (mJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>4.24 (0.14)</td>
<td>−20.93 (0.52)</td>
<td>−0.61 (0.02)</td>
<td>8.02 (0.11)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.23 (0.05)</td>
<td>−23.25 (1.11)</td>
<td>−0.48 (0.01)*</td>
<td>6.87 (0.47)</td>
<td></td>
</tr>
<tr>
<td>Stimulated</td>
<td>3.98 (0.13)</td>
<td>−40.23 (0.75)**</td>
<td>−0.72 (0.01)**</td>
<td>17.41 (0.11)**</td>
<td></td>
</tr>
</tbody>
</table>

Mann-Whitney U-test: *P < 0.028 vs. Basal; †P = 0.009 vs. Control; ‡P = 0.047 vs. Basal; †‡P = 0.009 vs. Basal.

---

Fig. 6. (a) Implant losses per week as a function of healing time. Both right and left implant losses are reported [total = 23]. (b) Implant losses at 6 weeks with respect to the external load [unloaded control implants not included].
force application to the (inherent) loads generated during daily activity. To this end, both tibiae were implanted simultaneously and thus prevented the rats from unloading the operated limb. Hence, it was the combination of both effects that affected implant integration. With respect to the levels of external stimulation, the 5N force induced a deformation of the peri-implant bone bed that was definitely in the upper range of the physiological strains arising in rat tibiae during gait [1000 με [Rabkin et al. 2001]–3300 με [Piccinini et al. 2012]]. This environment produced a broad palette of effects, that is, from implant loss to improved integration. These will be discussed hereafter in separate sections. The reliability of the FE model is also examined.

**Implant loss**

Figure 6a lists the implants lost and their respective integration period for both stimulated and control groups. With simultaneous bi-lateral implantation, both legs carried the animals’ weight during locomotion, and with reference to our previous study [Piccinini et al. 2014], it might be inferred that the absence of rest negatively influenced implant integration during healing. Indeed, the press-fit at surgery granted initial stability, yet five implants were already lost before stimulation. During the stimulation phase, loads applied to “weakly” integrated implants further deteriorated the site and drastically augmented the number of implants lost. As shown in Fig. 6a, losses peaked during the first and second weeks of stimulation (weeks 3–4). Stimulated implants were mostly affected.

As shown in Fig. 6b, load level and implant loss were not linearly correlated. Interestingly, the groups subjected to slight-to-moderate overloading (2N, 3N, 4N) were also those in which most implants were lost. These observations are at variance with those in which most implants were lost. The difference between those of previous test campaigns using the “loaded implant” model in which sites subjected to overloading (2N, 3N, 4N) demonstrated enhanced implant integration, in particular with respect to pullout force [Zucchetti et al. 2013]. As the difference between both regimens is bilateral implantation, it may be postulated that implanting both legs simultaneously alters the interaction of daily activity and external stimulation and largely deteriorates implant integration. These data confirm the strong sensitivity of this animal model to the stress fields generated during rat locomotion [Piccinini et al. 2014], as well as the relevant effect of external implant loading.

Table 3. Bone mineral density (gHA/cm³) in peri-implant regions of interest (ROI) (Fig. 1a)

<table>
<thead>
<tr>
<th>Group (n – 5)</th>
<th>ROI 1</th>
<th>ROI 2</th>
<th>ROI 3</th>
<th>ROI 4</th>
<th>ROI 5</th>
<th>ROI 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>0.542 (0.021)</td>
<td>0.604 (0.016)</td>
<td>0.548 (0.005)</td>
<td>0.439 (0.012)</td>
<td>0.739 (0.016)</td>
<td>0.802 (0.023)</td>
</tr>
<tr>
<td>Control</td>
<td>0.656 (0.031)</td>
<td>0.615 (0.012)</td>
<td>0.539 (0.020)</td>
<td>0.525 (0.021)</td>
<td>0.778 (0.027)</td>
<td>0.741 (0.027)</td>
</tr>
<tr>
<td>Stimulated</td>
<td>0.813 (0.023)*</td>
<td>0.626 (0.037)</td>
<td>0.728 (0.028)**†</td>
<td>0.456 (0.029)</td>
<td>0.705 (0.017)</td>
<td>0.789 (0.013)</td>
</tr>
</tbody>
</table>

Mean (standard error of the mean). U-test: *P = 0.014 vs. Basal; †P = 0.028 vs. Control.

Among the factors affecting implant integration (i.e. material, surface texture, surgical protocol), implant stiffness and geometry are yet other determinants of integration. Indeed, stiff implants can generate highly heterogeneous stress fields due to the interplay between stress-concentrating and stress-shielding effects. Moreover, the plain cylinders used in the present study provide no macrofeatures (such as screw threads) for anchorage in the surrounding bone bed. Their osseointegration may thus be compromised even under physiological conditions. Conversely, plain cylinders render the “loaded implant” model more discriminating and increase its sensitivity to external mechanical stimulation.

In this regard, when considering that the implants that survived best were those that adapted to the 5N external stimulation in addition to the loads imposed by the daily activity, it might be suggested that, in the present setup, the external stimulations counterbalanced the harmful effects of the daily functional loads. Indeed, according to Frost’s mechanostat theory, external loading at 5N would be expected to cause bone resorption due to damage accumulation. Yet such a response was not observed in the 5N specimens whose integration remained intact. This stability can only be explained by an additive-subtractive effect between the tensile strains due to gait [-1200με] and the compressive strains due to external stimulation [-3300 με].

**Enhanced integration**

As shown in Table 1, the groups that underwent mild stimulations (2N, 3N, and 4N) were those in which most implants were lost – an observation from which a detrimental effect of mild overloading was surmised [Fig. 5b]. This was the reason for removing these specimens from further consideration in the study. Besides, the specimens in which various degrees of cortical troughing occurred were not suitable for comparisons due to the uncontrolled variations in implant integration which in turn affected lever length and the response to external stimulation.
Thus, the present analysis focused on the specimens that had well integrated: five “basal” specimens, five specimens stimulated at 5N, and five “unstimulated controls” were randomly chosen among those obtained from the left tibiae. The implant heads of these specimens were forced together until failure to determine the inter-implant “ultimate” properties (Fig. 3). During these tests, it was always the osseous support of the proximal implant that fractured (Fig. 8a). Typical force-displacement plots for specimens belonging to the three groups are shown in Fig. 8b.

There is a striking difference between “basal” specimens and “unstimulated controls” – the latter demonstrating higher strength and less displacement. The origin of this observation is not known. It may be a normal effect of aging (Turner 2006) or due to a form of stimulated remodeling due to the presence of the implant. In this regard, note that the mere presence of an implant affects the stress fields at the site during normal daily activity. Interestingly, no difference between both groups was observable upon µCT analysis.

In contrast, the external activation to 5N definitively improved the mechanical resistance of the specimens, resulting in an increase of ultimate strength by 73%, displacement (+50%), and energy (+153%) in comparison with the “unstimulated control” group (Table 2). It thus appears that the bone adapted to external loading by augmenting both ultimate strength and displacement (note the difference with the “unstimulated control” in which the displacement lessened and thus embrittled the bone). A decrease in inter-implant strain (at 5N) was observed in the stimulated specimens (~6% – not statistically significant). The significance of this dichotomy [increase in ultimate strength, slight decrease in strain] is not known but is in agreement with studies on rat fibula subjected to mechanical loading (Robling et al. 2002). The effects of external stimulation were also assessed in terms of the BMDs of the ROIs (Fig. 2). In ROI-1 and ROI-3, BMD was significantly increased in the stimulated specimens relative to the two other groups. The medio-lateral ROIs and the bone tissue outside the implants (i.e. ROI-4 and ROI-5) were not subjected to significant BMD variations.

These data thus clearly indicate that the bone tissue adapted to the external load by increasing peri-implant BMD. Interestingly, bone remodeling took place both next to the distal and the proximal implants in spite of them being implanted either intramedullary or transcortically. Variations in BMD were observed along the tibiae’s longitudinal axes (i.e. the loading direction) but not in the medio-lateral ROIs. Moreover, a significant increase in BMD was measured only between the implants, where the bone tissue was subjected to compression during activation, but not in the tensile zones. µCT images of typical stimulated and unstimulated control specimens are shown in Fig. 9a, b. This load-dependent adaptation of the peri-implant bone resulted in a notable increase in ultimate mechanical strength and improved the implants’ resistance to vastly augmented stresses. It should be pointed out, however, that the specimens under scrutiny here were those that had well integrated and were not affected by the harmful effects of daily activity (i.e. implant disintegrations or cortical bone loss). Still, as such, they are indicative of the potential for the adaptation of a successful implant under critical mechanical conditions.

**Numerical modeling**

The computed local variation in BMD along the inter-implant plane of the five FE models of basal specimens subjected to 5N load is represented in Fig. 10. The first and second specimens from the left show small signs of apical resorption – possibly due to overloading (dotted circles). The BMD increments are mostly located in the sub-cortical inter-implant tissue in the vicinity of both implants. The magnitude of the increment, though, is specimen-dependent.

There was no effect on the bone tissue distal to the distal implant, thus validating the boundary conditions set to prevent unrealistic transmissions of tensile stresses (Fig. 5). Conversely, these boundary conditions also yield inaccuracies proximally to the proximal implant (dotted squares in Fig. 10), where augmentations in BMD appear. It appears that the numerical model of the trabecular reticulum surrounding the proximal implant relocates the anchorage point along the implant axes, thus introducing an unrealistic bone adaptation in this zone. Still a broad reading of these figures clearly indicates the local effects of the external stimulation in the form of significant increases in BMD between both implants.

The variations in BMD of the six ROIs are shown in Fig. 7a, in which both numerical predictions [for each specimen and their mean] and experimental data are presented. The experimental variation in BMD is taken as the difference between the values obtained in the “basal” and in the “stimulated” (5N) specimens (Table 3) that corresponds to the BMD evolution due to implant stimulation. Note that the numerical model replicates the local increases in BMDs (in agreement with the experimental data). The highest average increments are predicted for ROI-1 (0.4 gHA/cm³) followed by ROI-3 (0.35 gHA/cm³). The other ROIs show negligible variations in BMD. In line with the above, the increments in ROI-4 (which have no experimental match) are the consequence of the boundary conditions set in the present model (Fig. 6).

There is an important biodiversity in animal experiments which explains the important variances in the ROIs to the extent that their magnitude may be as high as the measurement itself [e.g. 0.3 gHA/cm³ in ROI-3]. Still, when considering the means, the comparison with the numerical values is satisfactory. The numerical model predicts the correct hierarchy of increments in the ROIs while overestimating the mean increase in BMD by a maximum of 0.14 gHA/cm³. This may be considered a quite acceptable result, considering the inherent biological dispersion and the number of assumptions made to model such complex biological phenomena.
The reasons of the overestimation are theoretical and experimental in nature. First, the numerical model assumes a location- and tissue-invariant limit for the assignment of BMDs. As a consequence, fully mineralized tissue (i.e. 1.2 gHA/cm³) may “appear” everywhere in the FE mesh if the appropriate stimulation is provided. Although this approach is taken by the majority of researchers, no experimental confirmation is available. Second, the experimental variation in BMD is taken at a defined time-point (i.e. 4 weeks after the onset of stimulation). Although previous studies on the “loaded implant” model indicate that longer stimulation periods do not substantially affect peri-implant morphology [De Smet et al. 2005; Wiskott et al. 2012], the new bone generated in reaction to the external loading is still in the process of mineralizing (and thus is not registered during μCT analysis). Hence, the BMD measured at 4 weeks of stimulation may be lower than the condition at equilibrium.

With respect to inter-implant stiffness, Fig. 7b compares the calculated variation of inter-implant strain compared to that measured experimentally [Table 2]. The bone’s adaptation to the external stimulation induces a reduction of inter-implant strain (i.e. increased stiffness) with notable differences between specimens (i.e. from −7.0 to −3.9%).

The average numerical result is in agreement with the experimental data although an overestimation of ca. 0.6% is noted. There is an interesting observation to be made in connection with this overestimation. Indeed, as the FE model indicates larger BMDs than in reality, concomitantly one would expect a larger decrease in inter-implant strain. Yet this is not the case (Fig. 7b). One possible explanation is that our numerical model does not provide for variations in bone geometry in the form of extra-cortical bone apposition. Should such phenomena occur, they will affect inter-implant stiffness but are not captured by the model. Conversely, as shown in Fig. 9, our numerical strategy allows internal bone adaptation such as cortical thickening by sub-cortical bone growth in compressive peri-implant regions [Fig. 9b, d].

The effect of biodiversity is substantial and impacts all output variables: the inter-implant strain and the global and local ROIs’ BMDs. As there is no criterion to a priori determine which specimen is representative of the whole population, data analysis might entail to substantially increase the number of animals or move toward individual-based bone adaptation laws.

The outcomes reported here might appear as contradictory to accepted theory. Further, to what extent they apply to intraoral conditions (in the context of chewing for instance) is another issue. As such, they should be considered basic research findings until their relation with clinical conditions is clarified.

Still this project shed some light on a number of aspects related to the mechanical overload of endosseous implants. These are summarized hereafter.

**Highlights**

Within the limitations of the study, the following statements are made:
Simultaneous bilateral implantation creates a "mechanically critical" environment as it is impossible for the animal to unload and thereby spare either leg. In the present experiment, the animals' activity induces significant tensile stresses and strains (~1200 microstrain) on the implant–bone interface which detrimentally affect the implant integration [Piccinini et al. 2014]. As a consequence, the implants' integration proceeds haphazardly and may fail even if only "mild" external stimulations are applied.

Conversely, strong compressive external stimulations (corresponding to ~5300 microstrain) counterbalance the harmful effects of daily activity. When applied to implants with appropriate primary stability, they lead to a significant increase of peri-implant BMD. The success of the integration is thus related to the interaction between tensile loading due to daily activity and the compressive loading applied externally.

Strong external stimulation significantly augments the ultimate inter-implant strength of well-integrated specimens relative to non-stimulated sites. The peri-implant tissue adapts to the loads imposed and provides a stronger anchor- age by localized increase of BMD.

A good agreement is found between the experimental results and the predictions of the proposed strain-based numerical model of bone adaptation. Although located in a wide range, the bone apposition and damage thresholds were in line with the literature [apposition range 1250–4150 microstrains]. The numerical predictions regarding the variations in BMD, both local and global, as well as the implants’ response to lateral loads, are trustworthy and the specimen-specific variations are properly captured.

Further validation of the numerical framework developed herein is the strategy to comprehend the process of implant overloading and assist in the development of therapies for bone augmentation and strengthening via controlled mechanical stimulation.

References


