Does milling one-piece titanium dental implants induce osteocyte and osteoclast changes?

Le meulage d'implants dentaires en titane entraîne-t-il des modifications du nombre d'ostéocytes et d'ostéoclastes?

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Summary

One-piece dental implants avoid adverse effects sometimes associated with the traditional implant-abutment interface and may provide a suitable alternative to two-piece implants; however, one-piece implants often need in situ milling, which may exacerbate cell apoptosis from excessive heat at the bone-implant interface and induce secondary crestal bone loss. Twelve implants were placed in the metaphyses of two sheep under general anesthesia. Six implants were milled with a diamond bur while the other six implants remained intact. Animals were euthanized after four days, and bone blocks were harvested. Bone samples were studied without decalcification. Osteocytes were stained with Hoechst 33342 and osteoclasts by the TRAcP reaction. Both cell types, in the cortical and trabecular bone around the implant’s cervical region, were counted utilizing morphometric methods. Values were compared to areas at a distance from the cervical region. No difference was observed between milled and unmilled implants, which suggested that the amount of generated heat did not provoke osteocyte loss or induce osteoclastogenesis. Intraoral abutment preparations did not increase cellular apoptosis at the bone-implant interface after four days in the ovine model.

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MOTS CLÉS

Implant monobloc ; Ostéocyte ; Chaleur ; Histomorphométrie osseuse

Résumé

Les implants dentaires monobloc évitent les effets indésirables rencontrés au niveau de l’interface implant-butée des implants dentaires traditionnels. Ils peuvent fournir une alternative aux implants classiques en évitant un deuxième temps opératoire ; cependant, les implants monobloc ont souvent besoin d’être fraisés in situ, cela peut, en théorie, induire l’apoptose cellulaire en raison de la chaleur excessive transmise à l’interface os/implant et pouvant conduire à une perte osseuse crestale secondaire. Douze implants ont été placés dans les métaphyses de deux moutons sous anesthésie générale. Les piliers de six
Introduction

Potential complications associated with the design of traditional two-piece implant-abutment connections include screw loosening, which can lead to interfacial joint opening, abutment micromovements and component fracture, structural weakness in small-diameter implant designs, and formation of an interfacial microgap between the implant and abutment, which may theoretically become a bacterial niche that can induce periimplantitis and secondary bone loss [1,2]. With one-piece implant designs, there is no interfacial screw to loosen, no implant-abutment interface that can cause structural weakness in smaller diameter implants (3 mm), and no potential for a subgingival microgap. Since 2004, one-piece dental implants have been commercially available from several manufacturers. The concept of a one-piece implant design is not new, however. From antiquity, a metal one-piece implant dating from the 1st century was found in a Gallo-Roman necropolis in Chantambre (France) [3]. During the 1960s and 1970s, one-piece implants made of titanium or zirconium were introduced by pioneers of modern implantology, such as Ledermann [4] and Sandhaus [5].

In 2004, a new, one-piece dental implant made of commercially pure titanium (c.p. Ti) (NobelDirect, Nobel Biocare AB, Göteborg, Sweden) was first introduced, and promising short-term results were published the following year [6,7]. Beginning in late 2005, several clinicians publicly raised concerns about unusually high bone resorption observed with one-piece implants, which they later documented, in the dental literature [8—10]. This triggered an investigation by the Swedish regulatory agency for medical products, which ultimately issued an alert to clinicians and recommendations to the manufacturer on protocol, instructions and required training for clinicians who use the one-piece implant. Conversely, subsequent publications by other clinicians have reported positive results with outcomes similar to those generally reported for two-piece implants [11].

Several pathophysiological mechanisms have been suggested to explain the excessive bone resorption observed in the earlier studies [8—10] such as the required in situ milling step used for height reduction of the one-piece implant [11]. It has been demonstrated in vitro that bone tissue damage can occur after heating bone to 47-50°C for one minute during osteotomy preparation [12—16]. In situ high-speed drilling for the placement of bone orthopaedic implants is a well-recognized cause of overheating followed by osteocyte death and subsequent bone loss [17—19]. Electrosurgery or drilling without coolant are also well known to have deleterious effects on alveolar bone [20,21]. The aim of the present study was to evaluate whether in situ milling of a one-piece implant would generate enough heat to induce cell apoptosis.

Material and methods

Implants

One-piece c.p. Ti implants from one manufacturer (Zimmer one-piece, Zimmer Dental Inc., Carlsbad, CA, USA) were selected for this study (Fig. 1). Implant surface morphology was verified by scanning electron microscopy (Fig. 2). The titanium implants were carbon-coated by evaporation (10 nm thick) with a MED 020 (Bal-Tec, Balzers, Liechtenstein) to provide the material surfaces with uniform electrical properties. Samples were examined with a JEOL 6301 F field emission scanning electron microscope equipped with an energy dispersive X-ray microanalysis machine (EDX-Link ISIS-Oxford). EDX was done by point analysis at the surface of the material to determine its composition. Images were taken with a five kilovolts acceleration voltage.

Surgical procedure

The experiment was conducted in a veterinary surgical school in accordance with ethical principles for animal studies and good clinical standards. The implantation procedure was conducted in animals in the same way that it is performed in humans. Two sheep of the Vendean breed, four to six years old, were the study subjects. Before surgery, animals were pre-medicated with an intravenous injection of benzodiazepam and the posterior limbs were prepared as for classical surgery. Natrium amoxicillin 500 mg was given by intravenous injection as a prophylactic antibiotic. General anesthesia was induced with intravenous perfusion of ketamine and, after an endotracheal intubation, maintained.
Bone cells close to milled implants

The one-piece titanium implant used in this study. A. Schematic drawing. B. Macroscopic aspect.

Histologic technique

After surgery, bones were immersed in an alcohol/formalin fixative for 24 hours at 4°C. Before removing implants, bones were radiographed on a numeric system (Faxitron Edimex, Angers, France) in order to determine the exact position of the implants. Implants were carefully unscrewed and blocks containing a single implant were sawn with a precision electric saw (Struers, Accutom-50, Copenhagen-Denmark). Contact radiographs of each block were taken (Fig. 4A) and blocks were processed undecalcified. Bones were dehydrated in absolute acetone for 72 hours at 4°C, immersed in acetone/xylene for 24 hours and defatted in xylene for 72 hours at room temperature. Blocks were embedded in purified methyl methacrylate in a cold environment [24].

Embedded blocks were dry-cut parallel to the implant axis using a heavy-duty microtome equipped with 50° tungsten carbide knives (Leica Polycut S, Rueil-Malmaison, France.). Sections were cut parallel to the long axis of the implant’s cervical region and centered on the maximal diameter (Fig. 4B). For each specimen, four serial sections (7 μm in thickness) were obtained at six different levels with a 50 μm space between each level. For each stack of sec-
Figure 2  Scanning electron microscopy of the one-piece titanium implant. A. The threads with a rough surface (bar = 100 μm). B. The abutment with a smooth surface (bar = 1 mm). C. The abutment of a retrieved implant which had been milled in vivo (bar = 1 mm).

Figure 3  Surgical view of two implants placed in the femoral metaphysis of a sheep. Vue chirurgicale de deux implants placés dans la métaphyse fémorale d’un mouton.

dations, one section was used for histochemical identification of osteoclasts by tartrate resistant acid phosphatase (TRACP) [25] and one for a precise identification of living osteocytes. Osteocytes were detected by an intense nuclear staining with Hoechst 33342 (2 mg dissolved in 1000 ml of distilled water, staining time: 30 minutes) and counterstained in red using nuclear yellow fast R (1 % in formalin containing phosphotungstic acid) during 30 minutes (Fig. 5) [26]. Hoechst 33342 has been used widely as a fluorescent dye for staining the nuclei of living cells [27]. It preferentially binds to AT regions of DNA and shows no cytoplasmic staining. Observations and measurements were made under UV fluorescence microscopy with a WU near-ultraviolet fluorescence cube on an Olympus BX51 microscope equipped with a DP71 digital camera.

Histomorphometry

Bone volume and osteocyte count were determined on Hoechst 33342 stained sections with an image analyzer (Leica Quantimet 550, Leica, Rueil Malmaison, France) with a specially written software in Quips™. The program measures the following parameters (the ASBMR—American Society for Bone and Mineral Research—nomenclature is used hereafter):

- the trabecular bone volume (BV/TV, in %) represents the amount of trabecular bone inside the cancellous space. It was measured after thresholding the trabeculae;
- the number of osteocytes (N.Ocy/B.Ar, in /mm²) was determined by automatically counting the number of osteocytes identified as highly brilliant spots inside the red trabecular bone matrix.

Microscopie électronique à balayage d’un implant en titane. A. Le filetage avec une surface rugueuse (bar = 100 μm). B. La zone avec une surface lisse (bar = 1 mm). C. La zone supérieure d’un implant prélevé qui avait été fraisé in vivo (barre = 1 mm).
Osteocyte number was determined in three different locations:

- the cortical bone region in direct contact with the implant abutment (an area a priori exposed with the maximum heat generated during milling);
- in the upper trabecular region close to the milling site,
- in the lower trabecular region at distance from the milling zone (Fig. 4C).

The number of osteoclasts (N.Oc/B.Ar; in \( \mu \)/mm\(^2\)) was determined similarly on TRAcP-stained sections. Only TRAcP positive cells in direct contact with the bone matrix were selected. Osteoclasts were only determined in trabecular bone.

**Statistical analysis**

Statistical analysis was performed using Systat\textsuperscript{®} statistical software release 11.0 (Systat Software, San Jose, CA). Differences among groups were searched with the Kruskal-Wallis one-way analysis of variance and differences between groups by Mann-Whitney’s U test when the Anova revealed a significant difference. Differences were considered as significant when \( p < 0.05 \). Results were expressed as mean ± standard deviation.

**Results**

TRAcP identification revealed osteoclasts as deeply stained cells with brown cytoplasms in direct contact with the blue calcified bone matrix. On Hoechst 33342 stained sections, osteocytes appeared as intensely stained greenish spots (and only intact nuclei were stained) on a red-stained background corresponding to the calcified bone matrix. Nuclei of bone marrow cells, endothelium and lining cells were also stained but did not interfere with measurements since the image of the trabeculae was used as a mask for counting osteocytes.

Results of histomorphometric analysis are summarized in **Table 1**. The trabecular bone volume was unchanged in both the upper and lower trabecular regions. No difference was also observed in the cortical bone. There was no increase in osteoclast number in the upper and lower trabecular regions, regardless of whether the implant had been

At distance from the implantation area, the region of interest used to obtain control values.

Figure 5  Histological aspects of bone cells revealed A. By the TRAcP staining which identify osteoclasts in purple, the bone matrix is lightly counterstained; original magnification × 100. B. Osteocyte nuclei identified by Hoechst 33342 with a red counterstain in fluorescence microscopy. Original magnification × 250. C. Same image treated by image analysis, the trabeculae are threshold and used as a mask to measure the bone surface, the bone marrow is eliminated. D. Same image after overimposition of osteocytes in pseudocolor.

Aspects histologiques de cellules osseuses après coloration. A. Par la détection de la TRAcP qui identifie les ostéoclastes en pourpre, la matrice osseuse légèrement contrecolorée ; agrandissement original × 100. B. Noyaux des ostéocytes identifiés par le Hoechst 33342 avec une contrecoloration rouge en microscopie de fluorescence. Agrandissement original × 250. C. La même image traitée par analyse d’image, les travées sont utilisées comme un masque pour mesurer la surface osseuse, la moelle osseuse est éliminée. D. Même image après surimposition des ostéocytes en pseudocouleur.

milled or not. In addition, the number of osteoclasts did not increase around the implants and did not differ from the mean value measured in the sheep femoral bone at distance from the implantation site. The number of osteoclasts was significantly higher in the deeper zones of the bone samples, at a distance from the cortice. Similarly, the number of osteocytes was not modified by milling in either the cortical area or the two trabecular regions. Living osteocytes were observed in bone trabeculae in the vicinity of the hole. On bone matrix debris, osteocytes were not identified in small particles but were present in larger particles intercalated with the trabeculae.

Discussion

One-piece dental implants could represent an interesting progression with the increased popularity of immediate loading. Several disadvantages of conventional two-stage implants are avoided: screw loosening, screw fracture,
Table 1  Results of histomorphometric analysis. 
Résultats des analyses histomorphométriques. 

<table>
<thead>
<tr>
<th></th>
<th>Unmilled</th>
<th>Milled</th>
<th>At distance</th>
<th>p</th>
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<tr>
<td><strong>Cortical parameters</strong></td>
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<tr>
<td>BV/TV (in %)</td>
<td>71.0 ± 5.3</td>
<td>62.6 ± 7.6</td>
<td>72 ± 8.3</td>
<td>NS</td>
</tr>
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<td>N.Ocy/B.Ar (in £/mm²)</td>
<td>232 ± 46</td>
<td>268 ± 75</td>
<td>253 ± 50</td>
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<td>BV/TV (in %)</td>
<td>26.4 ± 2.5</td>
<td>28.0 ± 2.0</td>
<td>27.2 ± 5.3</td>
<td>NS</td>
</tr>
<tr>
<td>N.Ocy/B.Ar (in £/mm²)</td>
<td>424 ± 117</td>
<td>438 ± 38</td>
<td>432 ± 50</td>
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<tr>
<td>N.Oc/B.Ar (in £/mm²)</td>
<td>9.4 ± 7.0</td>
<td>8.3 ± 4.4</td>
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<td><strong>Trabecular parameters, lower area</strong></td>
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<tr>
<td>BV/TV (in %)</td>
<td>21.3 ± 2.9</td>
<td>21.8 ± 4.9</td>
<td>22.1 ± 4.6</td>
<td>NS</td>
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<tr>
<td>N.Ocy/B.Ar (in £/mm²)</td>
<td>477 ± 96</td>
<td>457 ± 40</td>
<td>460 ± 68</td>
<td>NS</td>
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<tr>
<td>N.Oc/B.Ar (in £/mm²)</td>
<td>11.6 ± 10.5</td>
<td>8.5 ± 8.4</td>
<td>14.9 ± 9.5</td>
<td>NS</td>
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10,000/mm$^3$) [50]. The present method was found well adapted for bone cell morphometry. There was no difference in osteocyte number in cortical or trabecular bone in the vicinity of milled or unmilled implants. In the same way, the number of osteocytes was not modified when compared to areas at distance from the implantation hole. The four-day interval between implant placement and euthanasia was fully sufficient to induce cellular changes (if any) and sufficiently short to avoid spontaneous bone healing.

Drilling under coolant and milling the head of one-piece implants in situ with a bur did not induce osteocyte apoptosis or increase osteoclastogenesis in the vicinity of the implant during a short time period.

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References