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Original article

Osteoclastogenesis can be mechanically-induced in the peri-implant bone

La stimulation mécanique autour des implants peut induire la différentiation des ostéoclastes

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Abstract

Total joint replacements are highly successful in relieving pain and restoring movement of damaged joints. However, the lifespan of the implants is limited. The implant's long-term stability depends largely on the preservation of periprosthetic bone. Debris-wear particulates were first identified as the factor inducing periprosthetic bone loss. However, it was later shown that the resorption process starts before the particulates reach the periprosthetic bone. Thus a mechanical factor, interface micromotions, has been suspected to be the initiator of the early bone loss. In this work, we then investigated the response of bone cells to micromotions. Using an *ex vivo* setup, we applied micromotions on fresh human bone cores and showed that micromotions could indirectly activate osteoclasts after only one hour of stimulation. Thus micromotion-related osteoclastic activity could be the initiator of periprosthetic bone loss.

Résumé

Les arthroplasties totales des articulations permettent avec succès de diminuer les douleurs ainsi que de restaurer la fonction des articulations. Cependant, la durée de vie de ces implants est limitée. La stabilité à long terme des implants dépend largement de la préservation de l'os péri-implant. Des particules d'usure provenant des implants ont été initialement identifiées comme des facteurs induisant une perte osseuse autour des implants. Cependant, il a été montré par la suite que ce processus de résorption commence avant même que les particules aient pu atteindre l'os péri-implant. Par conséquent, un facteur mécanique, les micromouvements à l'interface, a été présenti comme étant l'initiateur de la perte osseuse initiale. Dans ce travail, nous avons donc étudié la réponse des cellules osseuses à des micromouvements. Utilisant une approche de type *ex vivo*, nous avons appliqué des micromouvements sur des cylindres osseux humains frais et avons montré que les micromouvements peuvent activer indirectement les ostéoclastes après seulement une heure de stimulation. Par conséquent, l'activité des ostéoclastes induite par des micromouvements peut être à l'origine de la perte osseuse autour des implants.

Keywords: Mechanical stimulation; *Ex vivo*; Human bone; RANKL; OPG

Mots clés : Stimulation mécanique ; *Ex vivo* ; Os humain ; RANKL ; OPG

1. Introduction

The components of a joint arthroplasty may become loose over time. The patient denotes increased pain in his replaced joint. On the X-rays, areas of loosening are identified by radiolucent zones revealing the replacement of bone-mineralized tissue by a soft fibrous tissue that does not provide the necessary mechanical support. Once a component becomes loose, it generally does not regain fixation in the future. For this reason, patients are followed on a regular basis with repeated radiographies to identify loosening risks.

The exact processes that provoke the replacement of periprosthetic bone by a layer of soft fibrous tissue are still under debate. Two hypotheses are generally used to explain the etiology of periprosthetic bone resorption. The most supported

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theory focuses on a biological reaction to sub-micron size wear particles. Numerous studies have shown that the debris after implant wear induces inflammatory reactions in the tissues surrounding the implant [1]. The second hypothesis suggests that mechanical factors also contribute to osteolysis. In particular, micromotions at the bone-implant interface are suspected to play a key role in tissue differentiation [2].

Wear at the articulating bearing surfaces can be defined as the removal of particles that occurs as a result of the relative motion between two opposing surfaces under load [3]. Total joint arthroplasty components are made of artificial materials. Over time, as these parts move back and forth relative to each other, this will result in wear of the components. Using radiostereometry, a very accurate measuring technique, Onsten et al. found a mean annual wear rate of hip components of 0.09 mm during a follow-up of five years [4]. Once removed from the implant material, the wear debris remain within the tissue and fluid surrounding the total joint arthroplasty. In tissue retrieved from hip revisions of 15 patients, Korovessis and Repanti found granules or larger cement particles, polyethylene fibers and metal deposits [5]. Previous studies have shown that these large amounts of wear particles of polyethylene, cement, metal or ceramics set into motion a cascade of cellular events in the periprosthetic bone [1]. The particles activate macrophages, which in turn trigger osteoclasts activity via pro-inflammatory factors [6]. The resulting imbalance in local bone metabolism leads to a progressive and massive bone loss.

The presence of an interface between two materials of different mechanical properties in a mechanically loaded structure induces a slip at the interface. In a joint arthroplasty, the slip between the metallic component and the bone is generally referred to as micromotions because of its magnitude: in hip replacements for example, relative displacements up to about 200 μm were measured at the bone implant interface during normal gait cycles [7]. Micromotion of the implant components relative to the adjacent bone in patients undergoing total joint arthroplasty are thought to contribute to aseptic loosening. In a dog model, Jasty et al. found that micromotions lower than 40 μm favor bone formation, while micromotions of higher magnitude lead to the creation of a fibrous tissue [8]. In a post-mortem study, Engh et al. found that cementless implants which showed signs of bony ingrowth had maximum relative micromotion of 40 μm, and implants which had failed bony ingrowth had relative micromotion of 150 μm [3]. Therefore, it is believed that micromotions may facilitate osteolysis by enlarging the so-called effective joint space [9] and hence allow access of debris particles to wider areas of the bone-prosthetic interfaces.

Rapid early migrations have been detected by roentgen stereophotogrammetry in many asymptotic hips, often as early as four months postoperatively [10]. Similarly, in knee replacements, Petersen et al. measured migrations of the component of 0.7 mm already after six weeks [11]. These early migrations of the components have been found to predict an increased risk of clinical loosening [12]. Furthermore, a clinical study showed that up to 14% periprosthetic bone is lost during the first three months after total hip arthroplasties [13]. And it was found that the early migration amplitude was correlated to the gravity of periprosthetic bone loss [11].

To sum up, these results indicate that the fate of an orthopedic implant is mainly determined at an early stage, probably before any wear particles are produced and can reach the periprosthetic bone. This suggests that early bone loss is related to other factors, for example mechanical factors. Micromotions at the bone-implant interface are then ideal candidates to explain the initiation of periprosthetic resorption. But the exact chronology of the early stage events that finally lead to critical bone loss is unknown. Therefore, we hypothesize that micromotions and compression at the bone implant interface may induce direct activation of bone resorption around the implant through osteoblasts-osteoclasts cells signaling in human bone. This hypothesis is tested with an *ex vivo* loading system using human bone samples.

2. Materials and methods

2.1. Bone samples preparation

Twenty-five human femoral heads were obtained from the "Hôpital orthopédique de la Suisse Romande" following total hip prosthesis procedures (Ethical Protocol 51/01, University of Lausanne). In the next four hours following the sample collection, each femoral head was fixed axially in a custom fixation device and at the central section a 6 mm thick slice was extracted with a surgical saw. Then, four to 16 trabecular bone cores of radius 3 mm and height 6 mm were extracted from the slice with a biopsy puncher (Shoney Scientific, Pondicherry, India) at 15 mm from the cortical bone layer (Fig. 1 a). The bone cores were

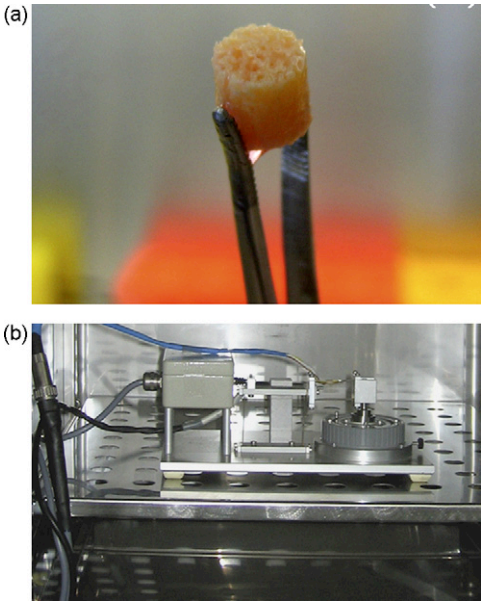

Fig. 1. a: bone core sample after being extracted from fresh human femoral head obtained at surgery; b: the bone core was inserted in the microstimulation device which was placed in a dedicated incubator during the 1 h mechanical stimulation. The upper part applies a 0.5 MPa static compression and 100 μm micromotions on the top of the bone core. The device is controlled with an external computer.

Table 1
Number of bone cores used for each condition.

No. of bone cores	Control	Compression	Micromotion
112	62	20	30

then incubated overnight in DMEM (Sigma, Buchs, Switzerland) containing 10% of fetal bovine serum (Sigma), and 1% of PSF (100X, 10,000 UI/ml Penicillin, 10,000 μg/ml Strepzin, 25 μg/ml Fungizone) (GibcoBRL, New York, USA) at 37 °C, 5% CO_2, 90% H_2O.

2.2. Bone samples stimulation

A device was developed to apply combined compression and micromotions regimen on the surface of trabecular bone samples simulating then the mechanical situation arising at the bone-implant interface [14]. Briefly, the device consists of a bottom fixed and top moving plates with bone core placed in between (Fig. 1 b). A 0.5 MPa static compression was applied from top and sinusoidal micromotions of 100 μm at a frequency of 1 Hz were applied on the top bone surface. The bone cores were separated randomly into three groups: control, compression and micromotions. Bone cores from the control group were incubated for 1 h at rest in 1 ml culture medium (control). Bone cores from the compression group were incubated in 1 ml culture medium and a static compression of 0.5 MPa was applied vertically on the sample in a special surgical steel chamber during 1 h (compression). Bone cores from the micromotions group were incubated in 1 ml culture medium. A 0.5 MPa static compression was applied from top and sinusoidal micromotions of 100 μm at a frequency of 1 Hz were applied on the top surface during 1 h (micromotion). The number of tested samples for each condition is given in Table 1. The parameters of the sample stimulations were chosen according to the results of previous numerical studies performed in our laboratory [15] and corresponded to a normal load during gait cycles.

2.3. Gene expression

Based on standard real-time RT-PCR analysis, the following gene expressions were quantified: RANK ligand (RANKL), osteoprotegerin (OPG) as target genes, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as non-regulated reference gene (housekeeping gene).

Relative gene expressions were calculated with the $2^{-\Delta\Delta CT}$ method [16] with GAPDH as housekeeping gene. We used a randomization of the differences and one-way ANOVA to compare the gene expressions of the different groups [17]. All values were then normalized to the expression of the control group. All mathematical operations and statistical analysis were performed using Mathematica® (Wolfram Research, Inc. USA). A p-value lower than 0.05 was considered significant while p-value lower than 0.1 was considered as a strong trend.

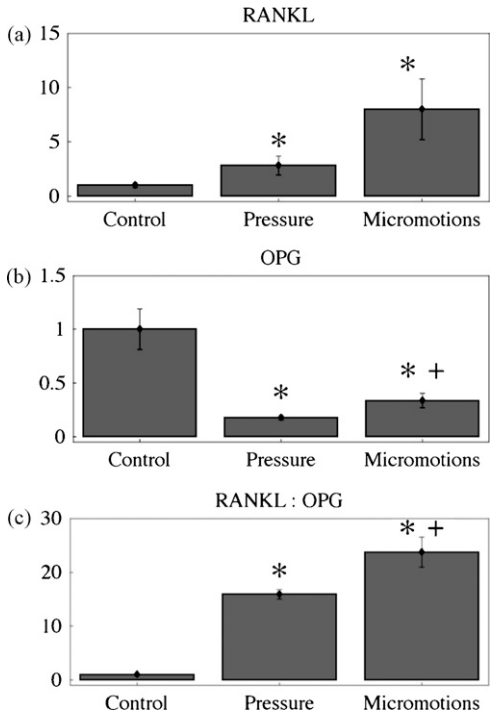

Fig. 2. Relative gene expression of (a) RANKL, (b) OPG and (c) the ratio RANKL/OPG quantified by RT-PCR after one hour of incubation (control), 1 h of 0.5 MPa static compression (pressure) or 1 h of 0.5 MPa static compression + 100 μm micromotion (micromotion). The results are shown as $2^{-\Delta\Delta CT}$ values and plotted as Mean ± SEM of each individual experiment. Symbols: * ($p < 0.05$ vs control), + ($p < 0.05$ vs static compression).

3. Results

In the following results, we report normalized gene expressions as Mean ± SEM.

RANKL expression was upregulated 2.8 ± 0.9 fold in the compression group and upregulated 8 ± 2.8 fold in the micromotion group when compared to control (Fig. 2 a). The difference between static compression and control, and between micromotion and control were significant, whereas there was a strong trend suggesting that the expression level between micromotion and static compression was different.

OPG expression was dramatically downregulated 0.18 ± 0.02 fold in the compression group and downregulated 0.34 ± 0.07 fold in the micromotion group when compared to control (Fig. 2 b). The differences between static compression and control, micromotion and control, and micromotion and static compression were significant.

Finally, the RANKL:OPG ratio was up regulated after micromotions compared to compression and control ($p < 0.05$) (Fig. 2 c).

4. Discussion

We hypothesized that micromotions could induce an upregulation of genes involved in osteoclastic bone resorption. Therefore, we analyzed the expression ratios of the RANKL/OPG signaling system. RANKL is a critical factor for late stage osteoclasts differentiation and activation. RANKL

was shown to be expressed by osteoblasts after mechanical or hormonal stimulations [18]. OPG, the decoy receptor of RANKL produced by osteoblasts is a powerful inhibitor of osteoclasts formation in vivo and in vitro [19]. Our results show that micromotion and static compression dramatically increase RANKL expression suggesting that the number and the activity of osteoclasts at the implant surrounding are increased by micromotion and compression in normal gait conditions. We also observed a down regulation of OPG expression after exposition to static compression alone or to micromotion suggesting that the balancing effect of RANKL by OPG is decreased.

We showed that the RANKL/OPG ratio is significantly increased ten fold by static compression and significantly more than twenty fold by static compression and micromotion, suggesting that micromotions are potent activators of high bone turnover rate. Our observations on the regulation of RANKL/OPG by static compression and micromotions suggest that the number of osteoclasts is enhanced, and bone turnover rate is increased in the periprosthetic area with normal gait cycle conditions. This might be one of the causes of the observed bone resorption around orthopedic implants.

The aim of the experimental setup used here was to simulate the mechanical situation at bone-implant interfaces using *ex vivo* bone samples. The amplitudes of the applied compression and micromotion were set to measured or calculated values. The drawbacks of this system are that the sample preparation procedures and *ex vivo* incubation certainly have biological consequences on bone cell functions despite we verified that at least there is an initial homogeneous level of gene expression between samples (data not shown). We can then assume that we have a consistency of initial conditions in our experiment. However, certainly due to the inherent biological variability, we obtained variable quality of extracted RNA, which may affect the gene expression quantification. It has to be mentioned that quantification of gene expression is only one part of the biological reaction to mechanical stimulus due to possible different post-transcriptional events. However, to our knowledge, no other experimental design allows one to study the effect of micromotion on the bone-implant interface with living human samples. These challenging technical difficulties were solved by a posteriori controls of RNA quality and variability of gene expression duplicates. More than 50% of the samples were discarded during these control procedures. It implied that paired-control statistical designs could not be used and that a large number of samples had to be processed to overcome the inter-specimen variations and to observe significant differences in the gene expression.

To conclude, our results suggest that micromotions at the bone-implant interface during normal gait cycles induce a rapid bone resorption response after only one hour, which occurs before any wear debris particles enter the system. These results confirm our initial hypothesis.

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