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Variation des propriétés biomécanique de l'os néoformé déterminées par nanoindentation en fonction du temps de cicatrisation

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Résumé :

Les implants en Titane sont utilisés de manière quotidienne en implantologie clinique et leur développement a permis des progrès en chirurgie dentaire et orthopédique. Après une intervention chirurgicale, un phénomène de remodelage osseux se produit naturellement conduisant à la formation d'os néo formé afin d'assimiler l'implant. Les propriétés mécaniques du tissu osseux situé à 100-200 µm de la surface de l'implant, sont déterminantes pour la qualité de l'ostéointégration et de la stabilité de la prothèse.

La stabilité d'un implant dépend des propriétés biomécaniques de l'os à son contact. L'objectif de cette étude est de déterminer les propriétés biomécaniques de l'os néoformé à différents temps de cicatrisation. Un modèle animal (tibia de lapin) permettant de distinguer l'os néoformé de l'os mature a été développé. Des mesures par nanoindentation ont été réalisées dans des échantillons d'os cortical néoformé (4 et 7 semaines) et dans l'os mature en parallèle d'études histologiques. Le module d'Young moyen obtenu pour un temps de cicatrisation de 4 et 7 semaines est égal à 14.80 GPa et 16.08 GPa et tend à retrouver les propriétés mécaniques de l'os mature (20.42 GPa).

Abstract :

Titanium implants are now routinely used clinically and their development have allowed considerable progresses in dental and orthopedic surgery. After a surgical intervention, remodeling phenomena normally occur, leading to the creation of newly formed bone tissue in order to accommodate the implant. The mechanical properties of bone tissue located at approximately 100-200 µm from the implant surface are determinant for the quality of osseointegration and the implant stability.

The aim of this study is to determine the biomechanical properties of the newly formed bone tissue at different healing time. A rabbit tibia model to distinguish the new formed bone tissue from mature bone tissue was developed. Nanoindentation measurements were performed in samples of cortical newly formed bone tissue (4 and 7 weeks) and mature bone tissue in parallel histological studies. The obtained Young's modulus average for a healing time of 4 and 7 weeks is equal to 14.80 GPa and 16.08 GPa and tend toward mature bone properties (20.42 GPa).

Mots clefs : Bone ; Implant ; Osseointegration ; Nanoindentation Measurements; Mechanical Properties

1 Introduction

Titanium implants are now routinely used clinically and their development have allowed considerable progresses in dental and orthopedic surgery. After a surgical intervention, remodeling phenomena normally occur, leading to the creation of newly formed bone tissue in order to accommodate the implant. The mechanical properties of bone tissue located at approximately 100-200 μm from the implant surface are determinant for the quality of osseointegration [1, 2]. A better understanding and characterization of the evolution of biomechanical properties of bone tissue at the microscopic scale may lead to more accurate prediction of implant osseointegration [3]. Such microscopic measurements can be done using different techniques. Scanning Acoustic Microscopy (SAM) has been used to assess bone acoustical properties *in vitro* with a resolution of around 30-60 μm [4]. More recently, a study published by our group [5] used a micro-Brillouin scattering technique to characterize the elastic properties of newly formed bone tissue. Nanoindentation is also likely to be used to retrieve hardness and Young's modulus at the microscopic scale. The aim of this paper is to investigate the biomechanical properties of newly formed bone in the vicinity of an implant using nanoindentation.

2 Material et methods

2.1 Implant

Two Titanium coin-shaped implants (5 mm \pm 0.05 mm in diameter and 3 mm \pm 0.05 mm in height) were used as described in details in [5]. These cylinders are made of medical grade titanium alloy (Ti-6Al-4V) and have their surfaces blasted with titanium dioxide (TiO₂) particles, leading to an average R_a value of 1.9 μm for the implant surface roughness. Polytetrafluoroethylene (PTFE) caps were placed around the implant in order to create a gap of 200 μm in height between cortical bone surface and the implant (see Fig. 1), 200 μm corresponding approximately to typical values obtained *in vivo* [6]. The gap of 200 μm allows bone formation to occur in a cylindrical volume (bone chamber 200 μm thick, 4.2 mm of diameter) and enables a clear separation between mature and newly formed bone tissue, which can be seen in histological analysis (see Figure 1). The implants were placed *in vivo* on rabbit tibia levelled cortical bone surface. Sous-sous-section (comme section)

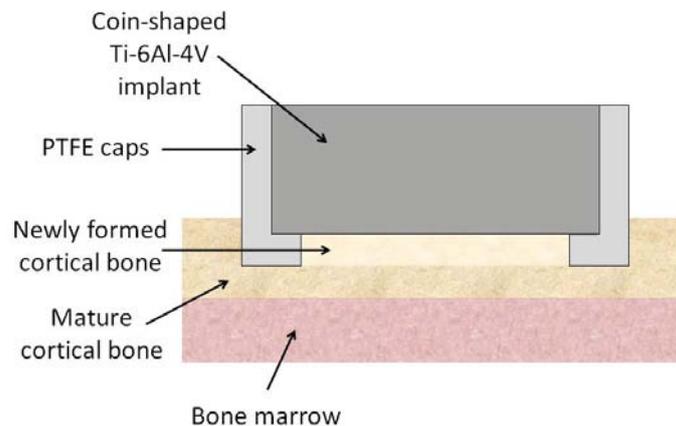


FIG. 1 Schematic representation of the coin-shaped implant model

2.2 Rabbit model

Two female New Zealand white rabbits were used in this study. The first rabbit was euthanized at 4 weeks (rabbit #1) post implantation and the second was sacrificed at 7 weeks (rabbit #2). The implanted tibiae were removed, prepared for histological analysis and cut perpendicularly to the implant interface. The procedure described in details in [7] for non-decalcified histology was used (samples embedded in PMMA).

2.3 Nanoindentation measurements

Nanoindentation was performed in mature and newly formed bone tissue using a nano indenter XP (MTS System Corp., Oak Ridge, TN) with a Berkovich tip and the continuous stiffness measurement (CSM) method [8]. The calibration of the nanoindenter geometry was performed with a fused silica sample. For each sample, indents were realized in mature and newly formed bone tissue. In rabbit #1, 132 (respectively 106) indents were realized in mature (respectively newly formed) bone tissue. In rabbit #2, 70 (respectively 64) indents were realized in mature (respectively newly formed) bone tissue. The locations where the indents (3 μm of maximal penetration) were realized are separated by a distance of 30 μm .

2.4 Statistical analysis

Statistical analysis (ANOVA test and Tuckey-Kramer analysis) were performed to investigate the difference of Young's modulus and hardness between samples for the different healing times. Statistical tests were performed using the Matlab software.

3 Results and discussion

Table 1 shows the difference in the averaged biomechanical properties between i) 4 weeks old bone tissue, ii) 7 weeks old bone tissue and iii) mature bone tissue.

	Newly formed bone 4 weeks	Newly formed bone 7 weeks	Mature bone
E _{harm} (GPa)	14,80 (± 0.95)	16,08 (± 0.85)	20,42 (± 0.98)
H _{harm} (GPa)	0,61 (± 0.05)	0,67 (± 0.06)	0,68 (± 0.06)

Table 1 Averaging results of the study

ANOVA ($p=5.61 \cdot 10^{-46}$, $F=145.71$) test reveals a significant effect of healing time on the Young's modulus of bone tissue. For hardness, the results are similar ($p=3.04 \cdot 10^{-2}$, $F=3.54$). A Tuckey-Kramer analysis revealed that the Young's modulus and Hardness values of newly formed bone tissue (4 weeks) were significantly different from those of mature bone tissue.

The results (Hardness, Young's modulus) obtained in this study are within the order of magnitude of results obtained in the literature. The difference of coloration in histological slide reveals a difference of mineralization between mature and newly formed bone, which may explain the difference in mechanical properties [9]. At four weeks, bone is less mineralized than after seven weeks of healing time. Biomechanical properties evolve during mineralization and tend toward mature bone properties.

4 Conclusions

A nanoindentation device was used to measure the Young's modulus and Hardness in mature and newly formed bone tissue, showing a significant effect of healing time. This study gives further insight on the evolution of biomechanical properties of newly formed bone tissue at the micrometer scale as a function of healing time. The results might lead to a better understanding of the clinical evolution of titanium implant.

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