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Longitudinal wave velocities in mature and newly formed bone tissue by micro-Brillouin scattering technique

顕微 Brillouin 散乱法による骨組織中の縦波音速測定

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1. Introduction

Osseointegration is an important phenomenon in the context of dental implant and orthopedic surgery. The quality of osseointegration depend on the changes in bone material properties and microstructure around the bone-implant interface (bone tissue located approximately at 100-200 μm around the implant) [1]. However, due to the complex nature of bone tissue and to remodelling phenomena, the evolution of implant stability remains difficult to assess and to anticipate [2,3]. Thus, it is of primary importance for the understanding of the osseointegration process to determine the characterization of the biomecanical properties of newly formed bone tissue in the vicinity of implants at the microscopic scale. In this study, we measured the wave velocities using a micro-Brillouin scattering technique (spatial resolution is approximately 10 μm) in order to investigate the potentiality of micro-Brillouin scattering technique to differentiate mature and newly formed bone tissue.

2. Material and methods

We used a coin shaped implant made of Ti-6Al-4V. A polytetrafluoroethylene cap was set around the implant so as to avoid bone growth around the implant and to create a 200 μm thick gap between the implant and bone surface, leading to the creation of a so-called *in vivo* bone chamber. Before setting the implant, irrigation holes were drilled through the bone leveled surface in order to allow blood discharge and stimulate bone remodeling in the bone-implant interface. The implant was included on the proximal part of a rabbit left tibia during 7 weeks. After sacrifice, the implanted tibia was removed and included in methyl methacrylate. After polymerization, the specimen was cut into slices along the implant axis and the slice containing the implant axis was chosen for micro-Brillouin scattering measurement. In order to obtain enough transparency to allow Brillouin scattering measurements, the thin sliced specimens were well polished down to a thickness of around 190 μm.

Brillouin scattering technique was performed by a six-pass tandem Fabry-Pérot interferometer using an argon ion laser (wavelength λ_0:514.5 nm). The micro-Brillouin scattering technique included a microscope for Raman scattering near the specimen. The actual spot diameter of the focused laser beam in the specimen was approximately 10 μm.

In this study, we employed the RIΘA scattering geometry shown in **Fig. 1** [4]. The geometry enables the simultaneous observation of the phonons propagating in each direction of wave vector of $q^{\Theta A}$ and q^{180} in one measurement.

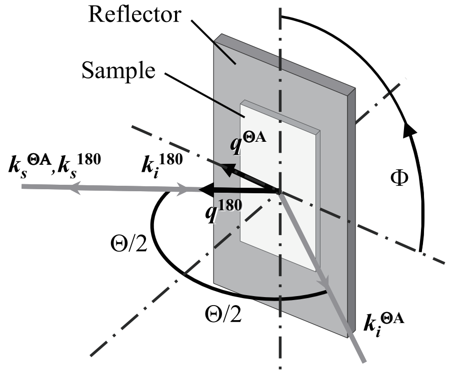

Fig. 1 The RIΘA scanning geometry.
$\boldsymbol{k}_i$ is the wave vector of the incident light, $\boldsymbol{k}_s$ the wave vector of the scattered light, $\boldsymbol{q}$ the wave vector of the sound wave, Θ/2 the angle between the incident laser beam and the normal line of the sample surface, Φ the rotation angle in the plane.

Velocity measurements were performed in newly formed and mature bone tissue in six different locations respectively.

A histological analysis was carried out after micro-Brillouin measurements so as not to affect bone biomechanical properties. The specimen was polished down to a thickness of 100 μm and then stained with Stevenels' blue and Van Gieson's Picro-Fuschin. Pigmentation enabled to discriminate newly formed bone from mature bone thanks to varying reactions to the degree of bone mineralization. Histological pictures were analyzed so as to confirm contents of the different volumes measured by micro-Brillouin scattering technique.

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3. Results and discussion

The wave velocity obtained from the 12 studied volumes, average and standard deviation are shown in **Table 1**. Wave velocity measurement error was approximately 1%. Higher average, minimum and maximum velocities are noted for mature bone than for newly formed bone. ANOVA (p=2.42 $\times 10^{-4}$, F=30.86) and Kruskal-Wallis (p=3.9 $\times 10^{-3}$, χ^2=8.31) show a significant difference between wave velocity measurements performed in newly formed and mature bone tissue. A Tukey-Kramer test with a 95% confidence interval shows a significant mean deviation between measurements performed in newly formed and mature bone tissue, which confirms the potentiality of micro-Brillouin scattering technique for the measurement of wave velocity variations between newly formed and mature bone tissue.

The use of Stevenels' blue and Van Gieson's Picro-Fuchsin histological pigmentations leads to bone regions with a crimson stain sensitive to the level of mineralization of the tissues. The more bone is mineralized, the darker the stain [5,6]. The histological image shown in **Fig. 2** indicates that the 200 μm thick bone chamber has partially been filled with bone tissue brighter than the surrounding mature bone. The brighter zone around the implant corresponding to newly formed bone tissue can be explained by the fact the mineralization process may not be completed after 7 weeks of implantation [7]. A lower degree of mineralization leads to lower values of elastic constants, which in turn leads to lower values of the wave velocity.

The wave velocity measurements performed in mature bone tissue were realized in locations distributed around the cortical layer of the specimen. In contrast, the wave velocities measured in newly formed bone tissue were in a region of interest of approximately 800 μm of diameter. However, the standard deviation of the wave velocity measured in newly formed bone tissue is significantly higher than in mature bone. This apparent contradiction may come from significant heterogeneity of newly formed bone tissue. Moreover, bone formation and remodeling might results from "streaming" phenomena arising from the irrigation hole (**Fig. 3**).

4. Conclusions

An *in vivo* surgical model implanting titanium coin-shaped implant in a rabbit tibia during 7 weeks was carried out. Micro-Brillouin scattering technique was used to measure elastic wave velocity in mature and newly formed bone tissue, showing a significant difference between the two groups of measurements. The results show that micro-Brillouin scattering technique is good candidate for the quantitative evaluation of the biomechanical properties of bone remodeled in the vicinity of an implant.

Acknowledgment

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Table 1: The wave velocity measured, Average and Standard deviation by micro-Brillouin scattering technique in newly formed and mature bone tissue.

Newly formed bone tissue		Mature bone tissue	
Measured location	Velocity [m/s]	Measured location	Velocity [m/s]
1	4.90×10^3	7	5.26×10^3
2	4.84×10^3	8	5.34×10^3
3	4.94×10^3	9	5.32×10^3
4	5.24×10^3	10	5.34×10^3
5	5.00×10^3	11	5.28×10^3
6	4.89×10^3	12	5.29×10^3
Average	4.97×10^3	Average	5.31×10^3
SD	0.14×10^3	SD	0.03×10^3

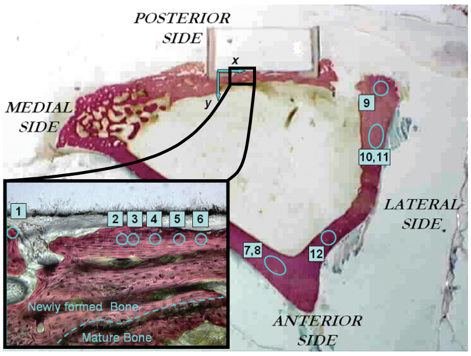

Fig. 2 The stained specimen and measurement point by micro-Brillouin technique.

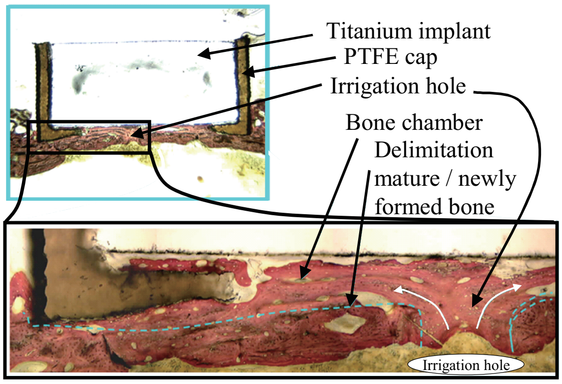

Fig. 3 Histological images measured in the vicinity of the implant.

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