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

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Brillouin scattering technique was performed by a six-pass tandem Fabry-Pérot interferometer using an argon ion laser (wavelength \(\lambda_0 = 514.5\) 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 \(\mu\)m.

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

Fig. 1  The RI\(\Theta\)A scanning geometry.

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 \(\mu\)m and then stained with Stevenels’ blue and Van Gieson’s Picro-Fuscin. 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 ($p=3.9 \times 10^{-3}$, $\chi^2=8.31$) 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 pigmentation 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 result 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.

<table>
<thead>
<tr>
<th>Measured location</th>
<th>Velocity [m/s]</th>
<th>Measured location</th>
<th>Velocity [m/s]</th>
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</thead>
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<tr>
<td>1</td>
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<td>Average</td>
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<td>SD</td>
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<td>SD</td>
<td>$0.03 \times 10^4$</td>
</tr>
</tbody>
</table>

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

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

References

3. Frost HM Anatomical Record Part a-Discoveries in molecular Cellular and Evolutionary Biology 275A(2) (2003) 1081-1101