Tissue and movement biomechanical characterization of osteoarthritis progression in mouse knee joint

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1. Introduction

In osteoarthritis (OA), tissue and movement biomechanics are both disturbed. Regarding movement biomechanics, OA induces gait disturbances such as increase in stride length (Poulet et al. 2015) and decrease of speed (Costello et al. 2010). Regarding tissue biomechanics, OA induces tissue degradation such as increase of subchondral bone (SB) stiffness (Zuo et al. 2016). However, there is a time-difference between tissue and movement biomechanics degradation in OA. The goal of this study is to investigate gait disturbances and to establish a protocol to test mechanical properties of SB in a mouse model of OA. First, we used an in vivo 2D gait analysis on living mice and second an ex-vivo Cantilever-based nanoindentation on femur SB.

2. Methods

2.1 Loading

Two groups of mice (Bl6C57 8 weeks old males) have been studied (OA group: n = 12, control group: n = 8). The tibia is placed between two cups (Figure 1) and a 9 N force was applied with a mechanical loading machine (ADMET Xpert 4000) (0.25s of rise and fall time, 2 N baseline for 9.9s, 40 cycles). Loading was applied 3 times a week for 2 weeks on the first group. This model is an adaptation of an existing loading OA model (Poulet et al. 2011) adding the rotation of 45° of the knee during the experiment. At the end of the two weeks, mice were sacrificed and right and left femurs were harvested.

2.2 Gait analysis

Gait of the mice was analyzed by recording their gait at a speed of 17 cm/s before the first loading, after 3 loadings and after 6 loadings. Data analysis was performed using DigiGait™ Imaging system (Mouse Specifics Inc., Boston, MA). Stride length and paw area were studied.

2.3 Sample preparation

Bone sample from a mouse from control group was embedded in PMMA and cut perpendicular to the bone axis, 1 mm deep in the condyle with a diamond saw in Phosphate Buffer Solution (PBS). This cut was polished with Silicon Carbide abrasive paper P1200, P2500 and P4000.

2.4 Cantilever-based nanoindentation

Nanoindentation was performed on SB of medial and lateral condyles and on PMMA using an atomic force microscope (AFM) (MFP-3D, Asylum Research – Oxford Instruments, Goleta, CA) with a diamond coated tip. Indentation modulus (E) has been calculated using the Oliver and Pharr method (Oliver and Pharr 1992):

\[
\frac{1}{E_r} = \left(1 - \nu^2\right) \frac{1}{E} + \left(1 - \nu_i^2\right) \frac{1}{E_i}
\]

Where \(E_r\) is the reduced modulus, \(\nu\) the Poisson’s ratio of the bone, \(\nu_i\) the Poisson’s ratio of the indenter, and \(E_i\) the elastic modulus of the indenter. \(E_r\) will be computed using:

\[
E_r = \frac{\sqrt{\pi} \cdot S}{2 \cdot \sqrt{A}}
\]

S is the measured stiffness and A the contact area.

The protocol of cantilever-based nanoindentation was established by Orestis et al. 2014. The maximum deflection used for our experiment is 2 V.
3 Results and discussion

3.2 Gait analysis

The results from the gait analysis are presented on Figure 2. Statistical analysis were performed with IBM SPSS Statistics 23 and p-value < 0.05 within each group. Friedman test was used to compare data. After one week and 2 weeks of loading, a significant decrease of the paw area was shown on the non-loaded leg. For both legs, after one and 2 weeks, a significant increase of stride length was observed. It shows that the loading experiment creates asymmetry in mouse motion. In previous studies, it was shown for the non-loaded leg that paw area and that the stride length increase after 2 weeks of loading (Poulet et al. 2015).

3.2 Cantilever-based nanoindentation

The indentation modulus (E) was obtained for PMMA and for bone (Table 1).

A value of 5.56 GPa was found for the indentation modulus, which is similar to Orestis et al. (2014).

The value for SB from control mouse bone sample is 12 GPa, which is in the range of what is found in the literature (Tai et al. 2007).

4 Conclusions

Our preliminary results show that two weeks of loading causes asymmetry in gait and our method of indentation allowed us to obtain values of indentation modulus for both subchondral bone and PMMA that make our protocol suitable to calculate subchondral bone stiffness.

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