ADVANCED CHARACTERIZATION OF THE 3D MORPHOLOGY AND MECHANICAL PROPERTIES OF THE ENTHESIS: OPTIMIZATION STUDY

Lisa Leyssens (1), Sophie Ryelandt (1), Audrey Favache (1), Greet Kerckhofs (1,2,3,4)

1. iMMC, UCLouvain, Belgium; 2. IREC, UCLouvain; 3. Dept. Materials Engineering, KU Leuven, Belgium; 4 Prometheus, KU Leuven

Connective tissue injuries occurring at the interface between soft tissue and bone, also called the bone-tendon interface or the enthesis, commonly result in disability and significant medical costs [1]. The chances of failure of surgical reattachment are high, which makes the bone-tendon interface an important research topic [2]. To address this clinical issue, it is necessary to regenerate a tissue that has the same properties as the original one. Before this is possible, it is important to understand the properties of the tissue that needs to be replaced. The purpose of this study was to develop and optimize advanced techniques to characterize the 3D structure and mechanical properties of murine entheses.

Achilles tendon-to-bone interfaces were extracted from 8-weeks old mice, 30-weeks old lean mice and 30-weeks old diet-induced obese mice. High-resolution X-ray microfocus computed tomography (HR microCT) and contrast-enhanced micro-CT (CE-CT) were used to determine the 3D structure and composition of the different tissues in the enthesis (figure 1). Nanoindentation was used to study the mechanical properties at the enthesis.

MicroCT revealed differences between the lean and the obese mice concerning the volume of the mineralized fibrocartilage layer and the surface of the connection between the mineralized and unmineralized fibrocartilage. Ageing did not influence these properties. CE-CT allowed observing the collagen fiber orientation within the unmineralized fibrocartilage and the connecting tendon. Quantitative 3D analysis is ongoing, as well as further optimization of the contrasting method. For the nanoindentation, we optimized the sample preparation protocol. Three sample preparation techniques were evaluated: (A) resin embedding and polishing, (B) resin embedding and cryostat sectioning, and (C) resin embedding and microtome sectioning. Method A showed unsatisfactory results because of the high occurrence of damaging the sample during polishing. In method B, the bone became very hard compared to the tendon at the low temperatures, making it challenging to cut the tissue without breaking the bone. Method C involved cutting a slice with a microtome. The remaining embedded part of the sample had a flat surface and was suitable for indentation: the biological structures were clearly recognizable and intact. With this preparation method, distinct mechanical properties could be detected for the different tissues within the enthesis.

Further research will involve the analysis of a large set of samples.

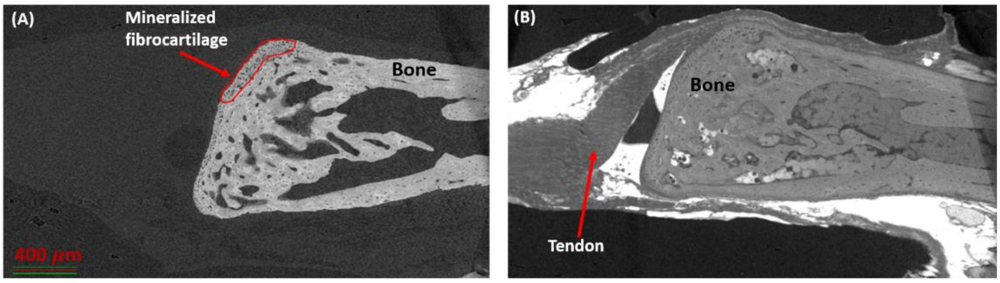

Fig.1: HR microCT image (A) and CE-CT image (B) of enthesis.

References:

[1] Lester Smith *et al*. Connective Tissue Research, 53(2):95–105, 2011.
[2] Guy M. *et al.* Biophysical Journal, 97(4):976–985, 2009.