NANOINDENTATION OF BIOLOGICAL TISSUES: OPPORTUNITIES AND CHALLENGES FOR THE BONE-TENDON INTERFACE

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Introduction

The bone-tendon interface is a highly specialized tissue connecting tendon to bone. The function of the bone-tendon interface is to mediate load between two very dissimilar tissues: the bone which is hard and stiff, and the tendon which is a soft tissue. Injuries occurring at this interface commonly result in pain, disability and significant medical costs [1]. These injuries are commonly caused by sports activities or can be seen in elderly people, making them less mobile because of the inability to move without pain. Moreover, the chances of failure of surgical reattachment are still very high. Therefore, the bone-tendon interface is an important research topic [2].

Nanoindentation is a powerful method for mechanical characterization of biological tissues [3]. It allows assessing the tissues at high resolution and in different directions, and it can be used to characterize complicated local mechanical properties of heterogeneous materials [4]. However, measurements remain challenging specifically for the bone-tendon interface because of the presence of highly dissimilar tissues. Moreover, sample preparation, which is a crucial step, is non-trivial for small biological tissues with highly heterogeneous properties. Therefore, the goal of this study was to optimize the sample preparation for nanoindentation of the bone-tendon interface at the site of the murine Achilles tendon-to-heel bone.

Materials, methods and results

Achilles tendon-to-bone interfaces were extracted from 30-weeks old lean mice. In order to perform nanoindentation, we aimed at obtaining a flat surface that showed both the tendon and the bone, so that the interface between them was also visible. Three sample preparation techniques were evaluated: (A) resin embedding and polishing, (B) resin embedding and cryostat sectioning, and (C) resin embedding and microtome sectioning.

In method A, epoxy resin embedding was done in vacuum and polishing was performed. This method showed unsatisfactory results. First, the embedding was challenging due to the difficulty of positioning the sample as desired (at the surface of the resin). Second, polishing was problematic because of impurities and bubbles in the resin, and because of the high occurence of damaging the sample. In method B, the samples were cooled down in ice for 15-20 minutes after embedding. Cutting was performed with a cryostat at -24°C. The problem with this method was that the bone became very hard compared to the tendon at these low temperatures, making it very hard to cut the tissue without ripping off the bone. Method C involved dehydrating the sample in ethanol prior to embedding in epoxy resin, followed by cutting a slice from the sample with a microtome equipped with a diamond saw. The slice was then placed on a glass slide. Nanoindentation could not be performed on the slice itself, as it was too thin (less than 1 µm thick). The remaining embedded part of the sample was placed in epoxy resin again, and as its surface was flat and suitable for indentation. As no polishing or cooling was required, better results were obtained compared to the other methods: the biological structures were clearly visible, recognizable and intact. With this preparation method, distinct mechanical properties could be detected for the different tissues within the bone-tendon interface (see figure 1).

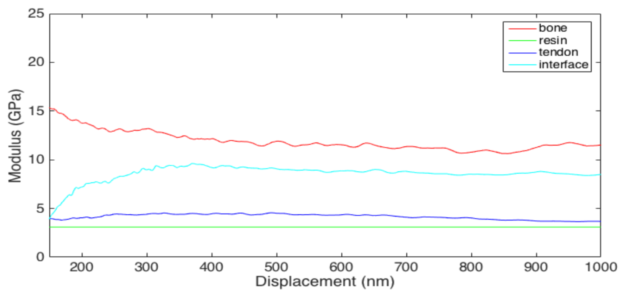

Figure 1: Elastic modulus of different regions of the interface obtained using *Agilent Nano Indenter G200*, method C, Berkovich tip

Conclusion

Mechanical testing of biological tissues is a critical step in the design and evaluation of replacing materials or implants, as well as in improving the understanding of certain diseases. The goal of this study was, therefore, to optimize sample preparation for nanoindentation of the murine bone-tendon interface. Further research will involve the analysis of a large set of samples in order to assess the effect of high-fat diet induced Type 2 diabetes on the mechanical properties of the bone-tendon interface.

References

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