Nanoindentation of biological tissues: opportunities and challenges for the bone-tendon interface

L. Leyssens[1], S. Ryelandt[1], A. Favache[1], G. Kerckhofs[1,2,3]

1 : Institute of Mechanics, Materials, and Civil Engineering, Université Catholique de Louvain, Louvain-la-Neuve, Belgium
2 : Dept. Materials Science and Engineering, KU Leuven, Leuven, Belgium
3 : Prometheus, Division of Skeletal Tissue Engineering, KU Leuven, Leuven, Belgium

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INTRODUCTION

Both for the design and evaluation of medical devices or implants/biomaterials and for the replacement of biological tissues, it is necessary to understand the biomechanical characteristics of the tissues to be replaced. Additionally, a change in the mechanical properties of biological tissues can be an important indication of a disease or a pathology. Mechanical testing of biological tissues is, therefore, a critical step in the design and evaluation of medical devices, as well as in improving the understanding of certain diseases. Biological tissues, however, have a non-elastic behavior, and thus there is a need for proper mechanical testing methodologies, set-ups and protocols.

The goal of this study was to evaluate the rate of change in mechanical properties within the transition region between the bone and the tendon, or the bone-tendon interface, which has a function of mediating 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 site commonly result in pain, disability and significant medical costs. The chances of failure of a surgical intervention can be very high, making the bone-tendon interface an important research topic. Characterizing its mechanical properties should, in the future, help to optimally reproduce the original tissue through tissue engineering to allow for successful repair (Smith et al. 2011).

The objective was to optimize the sample preparation for nanoindentation of the bone-tendon interface at the site of the Achilles tendon-to-heel bone in mice, which is only a few hundreds of microns long (approx. 200 μm), and to investigate the change in mechanical properties going from tendon to bone and correlate this change with the change in mineralization content and in collagen fiber orientation. Nanoindentation has recently emerged as a powerful method for mechanical characterization of biological tissues (Ebenstein and Pruitt 2006). 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 (Hauch et al. 2009). However, although sample preparation is a crucial step, it is non-trivial for small biological tissues with heterogeneous properties.

MATERIALS AND METHODS

Bone-tendon interfaces were extracted from mice and were fixed in 4% paraformaldehyde. The aim of sample preparation was to obtain a flat surface that showed both the tendon and the bone, placed in the correct direction so that the interface between them could be indented. Three main sample preparation techniques were evaluated: (A) resin embedding and polishing, (B) resin embedding and cryostat sectioning, and (C) resin embedding and microtome sectioning.

RESULTS AND DISCUSSION

In method A, epoxy resin embedding was done in vacuum. Polishing was performed using 1 μm grain size grinding paper. This method showed unsatisfactory results. First, the embedding was challenging due to the difficulty of positioning the sample as desired, so that it would be at the surface of the resin. Second, polishing was problematic because of impurities and bubbles that were entering the resin, and because of the high chances of damaging the sample. In method B, the samples were cooled down in ice for 15-20 minutes after embedding. Cutting could then be performed with a cryostat (*Leica CM3050 S*) at -24°C. Slices of different thicknesses were obtained ranging from μm up to 200 μm. 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 of 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 (*Leica EM UC6*) 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 in order to fill the remaining holes and pores and some slices were again cut from the sample. The remaining surface of the sample was entirely flat, making it possible to indent. As no polishing or cooling was required, better results were obtained: the biological structures were clearly visible, recognizable and intact. With this preparation method, distinct properties could be detected with nanoindentation.

CONCLUSION

Nanoindentation on biological tissues is a newly emerging technique and therefore, protocols for sample preparation still need to be optimized, especially for micrometer-scale, heterogeneous tissues. In this study, sample preparation for nanoindentation was optimized for the mouse Achilles tendon-to-bone interface, showing a change in mechanical properties.

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