DEVELOPMENT OF A MICRO TOMOGRAPHY PROTOCOL FOR PTA-CONTRASTED HUMAN MENISCUS

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Introduction

Contrast-enhanced micro tomography (CE-µCT) aims at enhancing X-ray contrast of low-density materials, mainly of soft tissues, allowing to investigate their 3D structure comprehensively. To promote CE-µCT as a standard technique complementary to immuno-histomorphometry, several aspects should be optimized: sample preparation and imaging; contrast agent diffusion together with minimizing tissue shrinkage; tissue preservation to ensure a not biased structural analysis and the complementarity with other assays [1]. In this study, a CE-µCT protocol on human knee meniscus with phosphotungstic acid (PTA) as contrast agent is under development by comparing it with chemical drying, a reference procedure to enhance contrast in biological samples, already experimented on human meniscus [2]. Indeed, PTA reveals preferentially collagenous structures [3], which are abundant in the meniscus, and it has been tested on animal models [4]. Nevertheless, replicating that protocol on human meniscal tissue highlighted only partial PTA diffusion, some tissue shrinkage and marked stiffening [5]. Therefore, an evolution of the protocol is under test.

Methods

Portions from human menisci (ethical approval 952/2021/Sper/IOR) underwent the following CE-µCT protocols. PTA concentration, originally at 1% (w/v) [4], was increased to 2% to improve diffusion; exposition to PTA lasted seven days. Water was compared to ethanol (EtOH) as solvent for PTA in terms of imaging. Samples were µCT scanned with source voltage 70 kV and current 250 µA, without metal filter (*Skyscan 1172*, *Bruker*, Belgium). As regards reference procedure – i.e. chemical drying – samples were dehydrated in ascending EtOH concentrations, treated with hexamethyldisilazane (HMDS), air-dried in a fume hood and then µCT scanned (40 kV, 250 µA; no filter) [2]. Sample dimensions before and after preparation for CE-µCT were measured by a caliper looking for shrinkage.

Results

Comparing sample contrasted by PTA in water respect to PTA in EtOH, meniscus radial section of reconstructed µCT images shows a major contrast, but a minor contrast agent diffusion; in both cases, signal appears highest on surface, corresponding to a major collagen density [2,4] (Fig. 1 A,B). HMDS drying resulted in minor image contrast (Fig. 1 C). Sample preparation caused tissue shrinkage in the case of chemical drying (-22% in volume change), not evident in the case of PTA contrast.

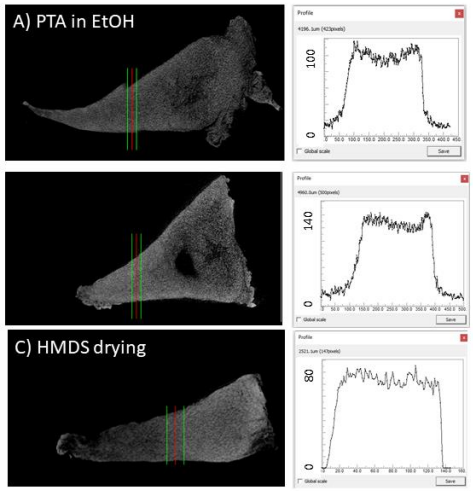

Figure 1: menisci CE-µCT sections and grey levels contrast profiles along red lines

Discussion

CE-µCT can be particularly useful for investigating the physiopathology of the human meniscus, important in knee biomechanics, often degenerated and still difficult to repair. We are developing a meniscus CE-µCT protocol based on PTA as contrast agent, investigating if it is possible to characterize collagen distribution and make indirect interpretations on the alignment of the extracellular matrix as for cartilage [6]. Validation is an important phase, often poorly described in relative literature: with this aim, histological and mechanical, i.e. functional, characterizations are ongoing [5], also testing rehydration/PTA washing strategies to favour the complementarity between those (and other) assays and CE- µCT.

References

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