Experimental report

25/01/2024

Proposal:	**8-05-477**	**Council:**	4/2023
Title:	Neutron imaging to quantify diffusion in knee joint tissues		
Research area:	Engineering		

This proposal is a new proposal

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Local contacts:	Alessandro TENGATTINI

Samples: tissue samples

Instrument	Requested days	Allocated days	From	To
NEXT	3	3	22/11/2023	25/11/2023

Abstract:

Water dynamics play a key role in properties of knee joint cartilage and meniscus. In order to improve understanding of these dynamics, we propose making use of neutron imaging¿s unique contrast between D2O and H2O to directly image diffusion. Bovine tissue samples will be repeatedly imaged over the course of ~12 hours while immersed in D2O from one or all directions. The diffusion front will be tracked and diffusivity quantified. The outcome will open up later possibilities for studying fluid dynamics inside the tissue during mechanical loading.

Neutron tomography of diffusion in knee joint tissues
Experimental report – D50 NeXT – ILL, Grenoble (11/2023)

Experiment No.: 8-05-477

A) Overview

Background: Water dynamics in soft tissue is an unexplored application of neutron tomography (NT). As water plays an important role in the load-bearing capabilities of joint tissues, understanding dynamics such as diffusion in joint tissues is important for research into osteoarthritis and other joint diseases. The unique ability of neutrons to detect contrast between H_2O and D_2O allow for direct imaging of water diffusion when one type of water is exchanged for another.

Aims: We aimed to directly image the diffusion of D_2O into H_2O-containing bovine knee joint tissue and evaluate how neutron imaging compares to other methods of measuring diffusivity such as MRI or QENS. A secondary aim was to explore the feasibility of in-situ loading of the same tissues.

***Method*:** NT imaging of bovine articular cartilage (AC) and meniscus tissues before and after being immersed in D_2O.

B) Experiment

Specimens: All specimens were sourced from bovine knee joints obtained from a local slaughterhouse. AC samples consisted of 5 mm round plugs drilled from the femoral condyle. Meniscus samples consisted of 5 mm plugs obtained from throughout the meniscus using a biopsy punch. All specimens were stored at -20°C prior to beamtime.

Diffusion experiment: Specimens were wrapped in PTFE tape leaving only the top surface of the tissue exposed. They were then placed in a bespoke PTFE sample holder, and a reference tomography taken (according to the scan parameters in table 1A). Afterwards, a D_2O-based saline solution was poured onto the samples. One sample of each type (AC and meniscus) was first radiographically imaged to gauge the speed of the diffusion process, and subsequent samples were imaged with consecutive tomographies. Due to the smaller tissue thickness (and correspondingly faster diffusion) of the AC samples, faster tomographies were used (table 1B). AC samples were also imaged with X-rays at the conclusion of the experiment. Reference tomographies of pure D_2O and H_2O were also taken as a point of comparison.

Mechanical pilot experiment: Shorter than expected measurement times allowed for a pilot experiment. Samples were placed in an aluminium tube in which mechanical compression could be applied via a screw. One meniscus and one cartilage sample were then treated by soaking in D2O for 12 hours and then imaged. One uncompressed reference tomography was then taken, followed by 2 tomographies following sequential compression steps.

C) Ongoing analysis

Images are being analysed to track diffusion fronts and quantify diffusion throughout the images. Complementary MRI scans are tentatively planned, as well as a proposal for a complementary QENS experiment.

D) Conclusions and feedback

During the beamtime we concluded from performed radiography that the planned 12-hour imaging times were more than necessary to track the diffusion front, and consequently imaged diffusion for 8 hours in meniscus and 2 hours in AC. Clear diffusion progression was visible in both types of tissue (figure 1).

The mechanical pilot experiment showed clear promise in mechanical testing, with water movement out of the tissue and into the permeable piston being clearly visible.

The support during the beamtime by Dr Alessandro Tengattini was excellent, we received both valuable advice on which imaging parameters to use and in performing image reconstruction. We would also like to thank technician Kalvin Buckley for excellent work in swiftly preparing sample holders.

Table 1. Image acquisition parameters.

A. Neutron tomography – Regular scans			
FOV [mm]	14x14	**Exp. time [s]**	0.5
Total rotation [°]	360	**Nmb. Projections**	900
Averaging	3	**Binning**	2
Pinhole [mm]	35	**Voxel size [μm]**	14.3
Imaging time [min]	22.5	**Reconstruction**	Absorption. RX Solutions X-Act Software
B. Neutron tomography – Fast scan			
FOV [mm]	14x14	**Exp. time [s]**	0.25
Total rotation [°]	360	**Nmb. Projections**	450
Averaging	3	**Binning**	2
Pinhole [mm]	35	**Voxel size [μm]**	14.3
Imaging time [min]	5.625	**Reconstruction**	Absorption. RX Solutions X-Act Software

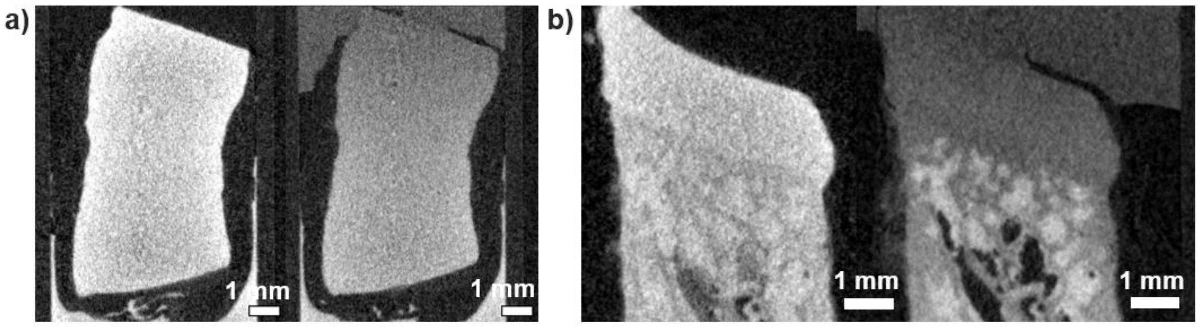

Figure 1. Meniscus (a) and articular cartilage (b) before (left) and after (right) the diffusion experiment. Exchange of H2O into D2O can clearly be seen in the tissue.

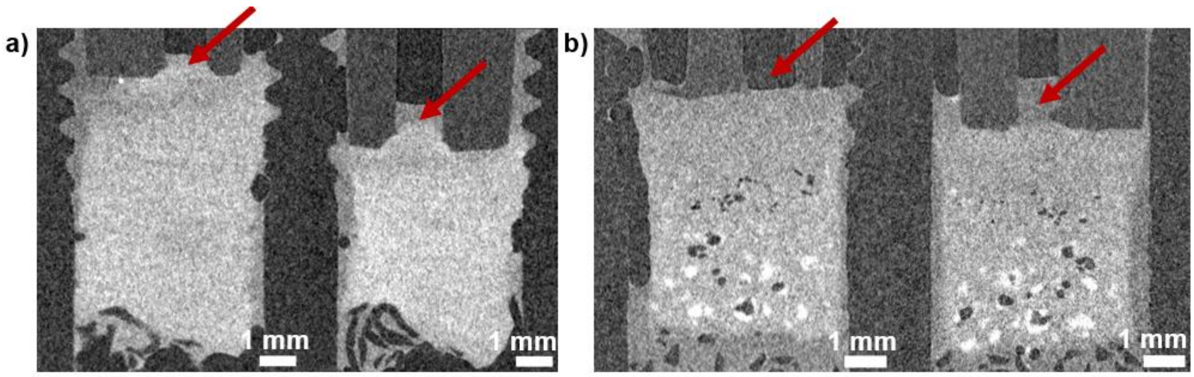

Figure 2. Meniscus (30% strain, a) and articular cartilage (10% strain, b) shown before (left) and after (right) mechanical compression. Water movement out of the tissue and into the permeable piston can be seen clearly (indicated by red arrows).