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PREVENTION OF CARTILAGE DEHYDRATION IN IMAGING STUDIES WITH A CUSTOMIZED HUMIDITY CHAMBER

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Introduction

Quantitative three-dimensional imaging methods allow rapid and comprehensive evaluation of cartilage and bone in animal models, used for drug development and related research in arthritis.

When imaging fresh cartilage tissue using synchrotron radiation microcomputed tomography (SR- μ CT), one problem is tissue dehydration causing movement artefact in the resulting images. This is especially noticeable at high resolutions (6 μ m and higher), and occurs even in fast (< 5 minute) scans. A humidity chamber was designed to counteract this problem and maintain cartilage hydration during scanning.

The goal of this work was to evaluate the performance of the humidity chamber. Quantitative measures of cartilage volume were made to test dehydration over scan time, and at various umidity (RH%) levels.

Materials and Methods

The humidity chamber holder houses a temperature/humidity sensor and heater that are used to monitor and regulate test conditions. Specimens are placed in a radio-translucent measurement tube which is screwed into the holder. This creates a seal which prevents moisture inside the chamber from escaping. The measurement tube is coated with lead film to protect the temperature/humidity sensor from radiation.

Three mouse knees were placed in the humidity chamber and scanned with SR- μ CT at the Swiss Light Source, using an isotropic voxel size of 3.7 μ m. The samples were immersed in 20% HexabrixTM (Mallinckrodt, USA), a staining agent to help visualise cartilage.

This study was divided into two experiments. Firstly, two knees were scanned using a low RH% for 20 minutes in 10 minute intervals to test the change in volume with time due to dehydration. Secondly, a knee was scanned at decreasing RH% of 97%, 92% and 87%, to test for a humidity level where volume drops away.

Bone and cartilage from the resulting images were manually segmented from the epiphyseal region of the tibia. Morphometric analysis was performed to determine the cartilage volume in each scan. Cartilage volume was then plotted against (1) time, and (2) RH%.

Results

Evidence of tissue dehydration is shown in fig. 1A; immediate loss of cartilage volume is observed when scanned at a low RH% (<90%) over a long period. In this scenario, dehydration occurs at approx 0.2 μ m/min. A loss of volume can also be attributed to scanning at decreasing RH% (fig. 1B).

Figure 1: (A) Cartilage volume against time for two mouse knees. (B) Cartilage volume against relative humidity for a mouse knee.

Figure 2: Overlay of the images at 87% and 92% relative humidity for a sample. The difference in cartilage thickness is shown by the grey border (indicated by arrows).

Discussion

The results clearly show that loss of cartilage volume is associated with scanning at low humidity (fig. 2). This effect can be avoided by using the humidity chamber for SR- μ CT imaging above 92%, since maintaining tissue hydration will reduce or eliminate cartilage shrinkage. Confirmation of these results is planned in ongoing work; since this study is limited due to restricted access to facilities for high-resolution SR- μ CT.

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