INTRODUCTION:
In-vivo evaluation of cartilage degeneration in small animals would be extremely useful for studying the aetiology and treatment of osteoarthritis. Currently, delayed gadolinium enhanced MRI of Cartilage (dGEMRIC) has shown to provide proteoglycan distribution by the diffusion of the contrast agent Gd-DPTPA in the cartilage, specifically at locations where loss of proteoglycans occurred [1]. A similar approach has been proposed for another ionic contrast agent (Hexabrix, Mallinckrodt) that could be used in combination with µCT [2]. µCT is nowadays widely used in osteoarthritis small animal research for studying changes in bone architecture and bone density. It would be of great benefit to combine both bone and cartilage µCT imaging in one longitudinal setting.

This study investigated the use of the contrast agent Hexabrix for µCT imaging of cartilage degeneration in small animals. Specifically we examined the spatial and quantitative sensitivity of contrast enhanced µCT for the glycosaminoglycan (GAG) content in articular cartilage. Furthermore, we evaluated its use in a time sequence of µCT images of a whole knee joint of a rat, where the Hexabrix slowly diffuses from the joint space into the cartilage.

METHODS:

In vitro assessment: 20 articular cartilage discs with a diameter of 6 mm were harvested from the meta-carpal-phalangeal joints of calves from a local abattoir. To accomplish differences in GAG content between samples, each sample was immersed in a different hyaluronidase solution, ranging from 0.0 - 1.0%. Hyaluronidase catalyzes the hydrolysis of hyaluronic acid, and with a decrease in hyaluronic acid, the GAG content of the cartilage will decrease. After the enzyme treatment, the discs were divided into three parts, used for: 1. Hexabrix enhanced µCT scanning.

3. Thionine (GAG) staining for digital densitometry (spatial assessment).

µCT scanning: prior to µCT scanning each sample was balanced in a 5 ml 20% Hexabrix x 320/PBS solution for 90 minutes. The scans were made with a skyscan-1172 µCT scanner at 10 µm voxel size.

Biochemical assay: GAG content of each sample was determined by using the DMB-assay. This GAG content was correlated with the mean attenuation values of the 3D reconstructed µCT images. The linear Pearson correlation coefficient was calculated.

Thionine staining: Vertical cryo-sections of 6 µm were obtained. The GAG distribution in the cartilage was visualized with a thionine staining that binds to the negative charged sulphate groups of the GAGs of cartilage. Photographs of the cryo-sections were matched with the corresponding µCT slices. A similar region of interest was selected from both the photograph and the µCT slice. (fig.1) Mean color density of each horizontal pixel row from the articular surface to the subchondral border was calculated. The Pearson correlation coefficient was used for statistical analyses.

RESULTS:
The GAG content determined by the DMB-assay was linear correlated with the attenuation value of the µCT (fig. 2a). Hyaluronidase caused a variation in distribution of the GAG content with much depletion in the top layer to less depletion in the bottom layer of the cartilage, as seen in the µCT slices and the thionine stainings (fig. 2b). The spatial distribution of the relative thionine density and the attenuation distribution of the corresponding µCT slices had a strong inverse correlation (fig. 2c).

DISCUSSION:
Delayed Hexabrix enhanced µCT is a highly sensitive method to monitor both absolute GAG content and cartilage GAG distribution. The results corresponded with both the quantitative DMB based GAG assay and the qualitative thionine based GAG staining. Although further in-vivo validation is needed, the delayed, intra-articular Hexabrix enhanced µCT of a rat knee gives detailed information about the morphology and thickness of the cartilage and other soft tissue structures, as well as the GAG distribution in the cartilage.

This method will be of great benefit in longitudinal imaging studies of osteoarthritic mice and rats. Based on our current in-vivo results, a much greater and faster penetration of Hexabrix is expected.

REFERENCES: