Introduction

Osteoarthritis (OA) is a progressive disease with limited treatment strategies. Therefore, there is a large need for testing disease modifying osteoarthritic drugs (DMOADs) in small animal models. For rats it is now possible to image cartilage using µCT-arthrography (µCTa) and determine its glycosaminoglycan (GAG) content, using a method similar to dGEMRIC \cite{1Peil et al. PNAS 103:19255, 2006}. The first goal of the current study was, to investigate how both quantity and quality of cartilage can be optimally analyzed from rat experiments using µCTa.

Second, we used the best analysis method to measure cartilage changes over time in different OA animal models. Three different rat OA models were selected, each representing a distinct OA etiology: running (‘wear and tear’ of cartilage during physical loading); monooiodoacetate (MIA) (increased apoptosis of chondrocytes with increasing age); groove model (local chondral trauma). The goal was to use µCTa in order to find specific OA dynamics for each model.

Methods

The institutional animal ethic committee approved all conducted procedures as described in this section. Six male Wistar rats received in one knee 1mg of MIA and contra-lateral a saline injection. After one week all knees were in-vivo scanned with µCTa, hereafter all patellas were harvested for an ex-vivo equilibrium scan (golden standard)\cite{2Piscaer, OAc 16:1011, 2008}. Three different global thresholds and one local threshold\cite{3Siebelt, M; Waarsing, JH; Piscaer, TM; Kops, N; Oei, E; Verhaar, JAN; Weinsans, H; Erasmus Medical University, Rotterdam, Netherlands} were used to analyze µCTa-data. Outcome measurements using these segmentation settings were compared for their structural representation with the ex-vivo golden standard.

With the best segmentation method from this first experiment, cartilage degradation was monitored in different models: running (strenuous running in a rodent treadmill, ±1.2km/day); MIA (intra-articular injection of 300μgr MIA, contra-lateral saline control); groove (trochlear cartilage was surgically grooved via surgery through the patellar tendon, contra-lateral sham). All groups consisted of six rats per group.

All knees were scanned with µCTa before OA-induction (t = 0) and at t = 1,3,6,12,18,24 weeks to monitor changes in condylar, patellar and trochlear cartilage. Afterwards, all knee joints were harvested for histology. Outcome measurements from µCTa were compared with unpaired-t-tests, histology averages were compared using non-parametric Mann-Whitney-tests.

Results

Cartilage quantity was best measured in datasets generated with local thresholds, that resulted consistently in the smallest measurement errors with least variation. Besides accurate structural representation, local thresholds also showed a significant increase of attenuation (less GAG) for MIA treated cartilage whereas global thresholds did not show any significant differences between MIA and saline control knees (figure 1).

Earliest signs of OA in the running model were seen after three and six weeks of follow-up in condylar cartilage, however, further OA progression was only minor. From twelve weeks up to the end of the experiment, trochlear cartilage showed more a pronounced increase in attenuation and volume decrease. For MIA injected rats, large increments in attenuation and loss of cartilage volume were measured for all anatomical regions. Grooved trochlear cartilage showed moderate signs of OA after surgery but lacked progression throughout the joint. Histology after 24 weeks of follow-up gave a similar representation of OA as detected with µCTa for all three models (figure 2).

Discussion

In-vivo µCTa-data can be used to closely monitor OA progression in small animals. Using local thresholds to segment the cartilage results in more accurate sequential measurements of both cartilage quality (GAG) and quantity (volume).

µCTa-data from the running model indicated that OA progression was ongoing in the whole joint even after the running stressor stopped, indicating that the ability for spontaneous repair was exceeded. This makes the model very suitable to monitor DMOADs designed to stop or inverse the OA cascade. GAG loss from cartilage throughout the entire knee joint can be seen directly after MIA injection. Therapeutic interventions trying to prevent chondrocyte death in OA as well as regenerative strategies can be well monitored in this model. The slow OA progression in local damaged cartilage in the groove model makes it an ideal candidate to study (cell-seeded/growth factor incubated) matrices designed to stop OA development from chondral lesions.

In conclusion, µCTa can monitor small changes in cartilage tissue and the wide implementation of the technique would serious enhance the ability to detect the effects from interventional strategies. Also, the OA dynamics outlined in this paper can contribute to plan and evaluate therapeutic studies.

References:

\cite{1Palmer et al, PNAS 103:19255, 2006}  
\cite{2Piscaer, OAc 16:1011, 2008}  
\cite{3Waarsing et al, J Bone Miner Res 19:1640, 2004}  
\cite{4Xic et al Osteoarthritis Cartilage 17:313, 2009}  

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