NOVEL BIOLOGICAL QUANTIFICATION OF MURINE OSTEOARTHRITIC JOINTS

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INTRODUCTION:

Articular joints are designed to withstand the dynamic and inertial loads of everyday activities, as well as providing stability to the body. When these joints are diseased they are often no longer capable of providing these functions. Osteoarthritis is one such disease and is characterised by cartilage degradation, bone changes and synovial inflammation. Extensive research is currently carried out on murine arthritis models in an effort to tackle this disease from a gene and cell therapy perspective. The mice are readily inbred to produce large numbers of genetically homogenous animals that can be used to study the pathophysiology of the early phases of osteoarthritis and to identify new targets for therapeutic modalities.

Spontaneous OA has slow onset and originates in, as yet, unknown genetic predisposition, whereas experimental induction of OA in animals makes it possible to influence the onset and the course of the disease [1]. However, in order to elucidate the nature of spontaneous OA, a model with naturally-occurring OA is desired. The highest incidence of naturally-occurring OA is reported in the STR/1N strain; where STR/ort mice are derived from this parent strain [2] and show a high occurrence in males. This mouse develops histopathological lesions in the medial tibial plateau similar to those in the human disease [3]. In this work, the STR/ort model is used.

In order to adequately assess the success of new therapies for OA, biological quantification is required. The aims of the research presented here are to provide tools for imaging which are specific to murine articular cartilage and subchondral bone. Novel techniques multiplexing confocal laser scanning microscopy with micro-computed tomography are developed and validated for volumetric and topographical imaging of articulating joints.

METHODS:

Male STR/ort mice were used as the OA model in this work, ranging in age from 3 to 10 months. The CBA strain was used as a control. The mice were sacrificed and the knees dissected free from surrounding tissue. The joints were imaged firstly with micro-computed tomography (µCT 40, Scanco Medical, Bassersdorf, Switzerland) with an isotropic voxel size of 12 µm. This provided image data of the mineralised, subchondral bone. The articulating surfaces were scanned through their depth using a CLS microscope (SP2, Leica Microsystems, Switzerland) with an isotropic voxel size of 6 µm, a 5x dry objective and a UV-laser light source (λ = 430 nm).

Images from both systems were processed using µCT evaluation tools. Cartilage tissue thickness (Cg.Th) and volume (Cg.V) were measured and compared across the joint surface. As were bone volume, tissue volume, trabecular thickness, spacing and number in the subchondral bone compartment.

RESULTS:

By utilising confocal laser scanning microscopy as a three-dimensional imaging tool, information about the quality of the articular surfaces can be gathered. The cartilage thickness from the lateral and medial plateau was firstly evaluated. Figure 1 shows the thickness of the medial and lateral plateau for the STR/ort strain. This figure highlights the loss of medial plateau cartilage in this region in the arthritic joints, a phenomenon not observed in the controls.

A comparison of the bone quality for young (3 months) control and STR/ort specimens show little difference (figure 2). Bone assessment of the ageing STR/ort shows a thickening of subchondral bone and an increase in osteophytes and bony fragments in the joints (figure 3).

DISCUSSION:

Mechanical and architectural changes to knee-joint subchondral bone have been shown previously in humans [4] and dogs [5], and now here in mice, and these changes have been quantified for healthy and arthritic specimens using micro-computed tomography. The results indicate that architectural alterations to the subchondral bone occur with the onset of OA. Alongside this, confocal microscopy provides a relatively fast means for gathering 3D image data on both healthy and diseased mice knees, providing a means to study small changes in cartilage thickness.

Using a novel multimodal imaging approach combining CLSM and µCT, a more complete picture of morphometric changes in the murine osteoarthritic knee joint can be seen; with further applications in other manifestations of arthritis in murine research.

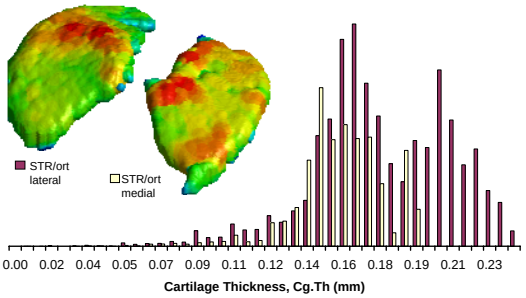

Figure 1: Cartilage thickness map and histogram of the lateral and medial plateaux in an arthritic murine strain.

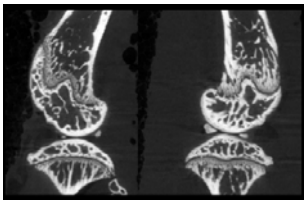

Figure 2: Three month-old CBA control (left) and STR/ort (right) joints.

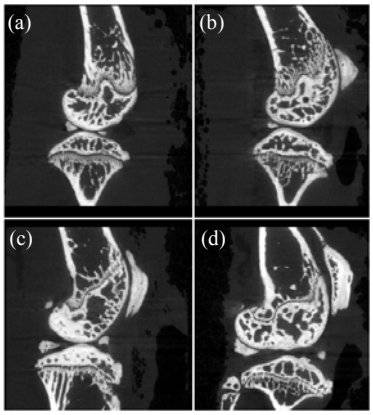

Figure 3: Changing bone morphometry with age, demonstrating increasing bone thickening and osteophytes; (a) 3 months, (b) 4 months, (c) 7 months, and (d) 10 months.

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