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Early in situ changes in chondrocyte biomechanical responses due to a partial meniscectomy in the lateral compartment of the mature rabbit knee joint

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ABSTRACT

We determined the biomechanical responses of chondrocytes to indentation at specific locations within the superficial zone of cartilage (i.e. patellar, femoral groove, femoral condylar and tibial plateau sites) taken from female New Zealand white rabbits three days after a partial meniscectomy in the lateral compartment of a knee joint. Confocal laser scanning microscopy combined with a custom indentation system was utilized to image chondrocyte responses at sites taken from ten contralateral and experimental knee joints. Cell volume, height, width and depth changes, global, local axial and transverse strains and Young's moduli were determined. Histological assessment was performed and proteoglycan content from the superficial zone of each site was determined. Relative to contralateral group cells, patellar, femoral groove and lateral femoral condyle cells in the experimental group underwent greater volume decreases ($p < 0.05$), due to smaller lateral expansions (with greater decreases in cell height only for the lateral femoral condyle cells; $p < 0.05$) whereas medial femoral and medial tibial plateau cells underwent smaller volume decreases ($p < 0.05$), due to less deformation in cell height ($p < 0.05$). Proteoglycan content was reduced in the patellar ($p > 0.05$), femoral groove, medial femoral condyle and medial tibial plateau experimental sites ($p < 0.05$). The findings suggest: (i) cell biomechanical responses to cartilage loading in the rabbit knee joint can become altered as early as 3 days after a partial meniscectomy, (ii) are site-specific, and (iii) occur before alterations in tissue mechanics or changes detectable with histology.

1. Introduction

Articular cartilage (AC) is a load-bearing tissue that covers the bone ends in an articulating joint. AC provides a smooth, lubricated surface to allow articulation and load transmission across the joint. The mechanical forces experienced by chondrocytes, the resident cells within AC, are known to modulate their biosynthetic activity (Stockwell, 1987; Guilak et al., 1997; Buckwalter et al., 2013) and thus, these cells are important in remodelling, regulating and adapting the joint tissue to meet the mechanical demands of the joint (Ramage et al., 2009; Bader et al., 2011). However, if chondrocytes can no longer maintain the integrity of the joint cartilage, the tissue may degrade. This process is commonly referred to as osteoarthritis (OA).

OA is often studied in animal models instead of in humans because of limitations including the availability of human tissue, the slow disease progression and the absence of symptoms (i.e. pain) during the early stages of the disease in humans (Lampropoulou-Adamidou et al., 2013). OA in human joints is often detected in the later stages of the disease, when a substantial loss of cartilage has already occurred (Lampropoulou-Adamidou et al., 2013; Lane et al., 2011). Creating mechanical instability and altering joint loading within the rabbit knee joint through the use of surgical procedures, such as a partial meniscectomy (PM) or an anterior cruciate ligament transection (ACLT), are common approaches used to study the progression of post-traumatic OA in animals (Bendele, 2001; Lampropoulou-Adamidou et al., 2013; Sanchez-Adams et al., 2014).

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PM or ACLT models of OA create altered knee joint biomechanics by increasing medial-lateral shear forces in the partial meniscectomized knee joint compartment (Minns and Muckle, 1982) or by creating abnormal anterior tibial displacement and internal tibial rotation during extension/flexion in anterior cruciate ligament transected knee joints (Nigg and Herzog, 2007). This leads to altered contact pressure distributions across the joint surfaces and, over time, causes knee joint cartilage to develop pathological features of OA (Minns and Muckle, 1982; Nigg and Herzog, 2007). Furthermore, identifying where and how the cell and tissue level changes begin to manifest are important in order to understand the development of post-traumatic OA in the rabbit knee joint.

Early changes in rabbit knee cartilage due to a PM have been shown to include a reduction in proteoglycan (PG) content within the tissue occurring as early as 2–3 days post-surgery (Colombo et al., 1980; Caputo et al., 1988). Macroscopic lesions on joint surfaces have been reported as early as one week post-surgery, with lesions spreading across the articular cartilage and producing more intense surface fibrillation 12 weeks after surgery (Colombo et al., 1980). In ACLT rabbit knee joints, visual signs of cartilage loss from joint surfaces have been reported as early as two weeks post-ACLT surgery (Oba et al., 2005) with the development of lesions and surface fibrillation increasing in severity by 8 weeks post-surgery (Bluteau et al., 2002). At 4 weeks after an ACLT surgery, site-specific alterations in cartilage structure, composition and mechanical properties have been found in rabbit knee joint cartilages (Turunen et al., 2013; Mäkelä et al., 2014; Florea et al., 2015), with PG loss observed across the load-bearing surfaces of femoral condylar articular cartilage (Arokoski et al., 2015).

Previous research demonstrated that biomechanical responses of superficial zone chondrocytes to indentation are site-specific within the rabbit knee joint (Fick et al., 2015), and that these biomechanical responses to indentation are altered 4 weeks post-ACLT in superficial patellar rabbit cartilage (Turunen et al., 2013). Furthermore, the aforementioned changes in chondrocyte responses have been attributed to changes in tissue PG content and disorganization of the collagen network (Turunen et al., 2013; Tanska et al., 2013). However, no study has examined chondrocyte biomechanics at various sites within the rabbit knee joint to determine if site-specific changes occur at an early time point following a PM.

Therefore, the purpose of this study was to determine if superficial zone chondrocytes from different locations within the knee respond differently to cartilage indentation loading as early as 3 days after a PM of the lateral meniscus. We hypothesized that a PM is associated with a reduction in tissue PG content and chondrocyte volume changes, in agreement with the changes observed at the initial stages of osteoarthritis (Han et al., 2010; Turunen et al., 2013). In order to test this hypothesis, we investigated chondrocyte deformations through the use of a custom-built indentation system (Han et al., 2009) attached to the stage of a confocal laser scanning microscope (CLSM). The tissue sites investigated were from both compartments of the femoral condyles and tibial plateaus, the patella and the femoral groove of New Zealand White rabbits. Changes in cell volumes, cell dimensions, global, local axial and transverse tissue strains, along with Young's moduli and Mankin scores were determined for each tissue site. Histological analyses were performed for each location in order to quantify the structure and composition of the superficial zone of cartilage.

2. Materials and methods

A brief description of the methods is given here; more details are listed in the Supplemental materials.

2.1. Sample preparation

Ten skeletally-mature female New Zealand White rabbits (age 13 ± 1 month) underwent a PM performed in the lateral compartment of a randomly selected knee joint (Fig. 1), forming an experimental (surgery) knee group ($n=10$). The non-operated knee joints formed the contralateral knee group ($n=10$). Rabbits were sacrificed three days following the surgical procedure and knee joint tissues were harvested. After tissue harvesting, each sample underwent confocal imaging before and during indentation loading, and then was placed into separate jars containing formalin fixative. The fixed tissues were shipped from Calgary, Canada to Kuopio, Finland for histological analyses.

AC-on-bone samples from the patella, femoral groove, lateral and medial condyles and lateral and medial tibial plateaus of each rabbit knee (6 sites/knee joint) were prepared for indentation and CLSM. Joint tissues were stored in Dulbecco's Modified Eagle's Medium (DMEM, Sigma Aldrich, USA) at 4 °C until required for testing. Before testing, all samples were stained with fluorescein conjugated Dextran and mounted into a sample holder such that indentation would occur in the targeted areas (Fig. 1). A total of 9–10 indentations were performed at each site in each group.

2.2. Confocal laser scanning microscopy experiments

Samples were placed into the chamber of a custom-designed in situ indentation system (Han et al., 2009), immersed in DMEM, and mounted to the stage of a confocal laser scanning microscope (LSM 510, Zeiss Inc.; Fig. 2). An imaging and loading protocol was used as described previously (Han et al., 2009, 2010; Turunen et al., 2013; Fick et al., 2015) in order to image 10 superficial zone cells from each joint location. A total of 90–100 cells from each site for each group were captured for further analysis.

The percentage change in mean cell volume and cell dimensions (i.e. height, width and depth) due to cartilage loading was determined for all tissue sites. Local axial, transverse (in both major and minor directions) ECM, global strains and Young's moduli were determined from each sample and then averaged across each location. For further details, see the Supplemental materials.

To determine if the contralateral tissue sites where appropriate to use as reference sites for this investigation, Schuirmann's Two One-sided tests were used to demonstrate equivalency (within ± 5%) with mean cell volumes determined for each site from the contralateral group and mean cell volumes from the same tissue sites taken from normal New Zealand White rabbits from a previous study (Fick et al., 2015). For more details, see the Supplemental materials.

2.3. Analysis of tissue histology, composition and structure

Tissue histology was determined by scoring tissue sections (via modified Mankin scoring). Further information on this and mean scores for each site from both groups are provided in the Supplemental materials. To further characterize the superficial zone composition from each site, proteoglycan content and collagen fibril orientation angle were also evaluated for each site from histological sections (3 sections per site) as previously described (Mäkelä et al., 2014) using digital densitometry and polarized light microscopy, respectively. Mean values for each parameter were calculated up to 10% of depth from each section, with mean values obtained for each sample and then for each tissue location. For more details see the Supplemental materials.

2.4. Statistical methods

For each parameter, the values were pooled according to site and group. Independent student's *t*-tests were utilized to compare the mean parameter values obtained for the groups amongst the different sites investigated ($p < 0.05$). All data are reported as means ± 95% confidence intervals (CI). All statistical analyses were performed using IBM SPSS Statistics 21.0 (IBM Corp, Armonk, NY, USA).

3. Results

3.1. Deformation induced changes in cell volume and dimensions

Fig. 3 demonstrates the changes in cell volumes and dimensions (height, width and depth) at the various sites from both contralateral and experimental groups due to cartilage loading.

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