Permeability of Calcified Cartilage in Osteoarthritis Joints

Introduction
Osteoarthritis (OA), a progressive disease of joint degradation, is characterized as loss of articular cartilage with associated soft and hard tissue changes. Nowadays, no effective treatment is available to cure OA after its initiation due to the complexity of the etiology [1]. There is increasing evidence from OA patients and animal models that subchondral bone turnover is significantly increased [2]. It was hypothesized that various cytokines and growth factors released during subchondral bone turnover may reach the overlying articular cartilage and initiate a vicious positive feedback between attempted cartilage and bone repair processes that eventually leads to OA progression [3]. To test the hypothesis, it needs to elucidate whether and how biofactors communicate or transport between subchondral bone and articular cartilage. The recent research from Arkill [4] and our group [5] have shown that, in contrast to the general belief, the calcified cartilage sandwiched between the subchondral bone and articular cartilage is not an impermeable barrier, and allows the passage of small tracers in normal adult joints. In the present study, we examined whether and how the solute transport characteristics are altered in OA joints. Using two OA (ageing and the surgical destabilized medial meniscus (DMM)) models and our newly developed fluorescence loss induced by photobleaching (FLIP) method [5], we quantified the diffusion coefficients of a small tracer within calcified cartilage and between calcified cartilage and subchondral bone in OA joints.

Methods

Mice: Three experimental groups of C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME) were included in this study. 1) Ageing induced OA mice. Four male C57BL/6J mice were housed and aged till twenty-month old. 2) DMM surgically induced OA mice. Following the approval of the Institutional Animal Care and Use Committee, four male C57BL/6J mice (3-month old) underwent DMM surgery as described previously [6]. Mice were anesthetized with isofluorane. The right leg was shaved, cleaned and the joint capsule was open to expose the medial meniscus. The ligament attaching the medial meniscus to the tibia was then transected to impair the stability of the joint structure [6]. The left leg was untouched and served as an internal control. After surgery, mice were placed on a warm pad and waked up in about five to ten minutes. Normal mobility was usually obtained in one or two days. The mice were housed for three and half months post surgery before being used in FLIP experiments. 3) Normal age control mice: Four C57BL/6J mice of 5-6 month old. Histological examination confirmed that OA-like cartilage degradation such as surface fibrillation and focal loss of proteoglycan staining occurred in the joints of ageing and DMM mice, while the cartilage of the normal age control showed normal proteoglycan staining and no obvious structural changes.

Quantitative Measurements of Solute Transport: The mice were injected with a bolus of sodium fluorescein solution (30 mg/mL, 0.5 mL) via a tail vein and sacrificed 25 min later. Fresh right distal femurs were harvested and the femoral distal ends (~2-3 mm) were sagittally split between two condyles and trimmed at ~40°C using a cryomicrotome (Leica, Wetzlar, Germany) equipped with a diamond knife (Diatome, Switzerland). Samples were mounted on a cover glass with the cutting surface facing the glass. Tracer distribution and overall morphology over the depth of femur epiphysis was examined using an inverted confocal laser scanning microscope (Zeiss LSM 510) with 488nm excitation. The individual chondron within calcified cartilage and 5-20 μm below the cutting surface were identified and subjected to FLIP as described previously [5]. Briefly, a chondron was chosen in the calcified cartilage region and subjected to high laser illumination (photobleaching), creating a sink in a previously equilibrated system and driving diffusive flux into this sink from the surrounding chondrons and osteocytes (sources). Using our hemispherical transport model [5], the effective diffusivity of the tracer (D) within the calcified cartilage between the source and sink chondrons (C-C) and between the source osteocytes and sink chondron (O-C) could be derived from the intensity decay in the surrounding cells (sources) as well as the size and spacing of the sources and sink cells. Usually 3-6 FLIP experiments were performed per animal and 2-4 pairs of source-sink were analyzed per FLIP experiment. The studies were performed on ageing and DMM OA joints and compared with results from the normal adult control mice [5].

Results
In all mice joints, the tracer D measured between chondrons in calcified cartilage (C-C) was significantly higher than that measured between osteocytes and chondron (O-C) in the same mouse group. However, there was no marked difference of C-C and O-C diffusivity among the three mice groups (Table 1). Compared with normal mice (Fig. 1C), aged OA joints showed a much wider distribution of C-C and O-C diffusivity (Fig. 1A), while the DMM OA joints appeared to have more C-C pairs with smaller diffusivity (Fig. 1B).

Discussion To our knowledge, this is the first paper quantifying the solute diffusivity in calcified cartilage of OA joints. Similar diffusivity of sodium fluorescein within calcified cartilage and between calcified cartilage and subchondral bone was found in normal, ageing, and DMM joints. These results suggest that the mineralized matrices in the two OA animal models may have similarly averaged transport characteristics as in the normal adult controls. However, we did observe that the variability of the local permeability in the calcified cartilage appeared to be altered in ageing OA and DMM surgical OA joints. In ageing OA joints, there was increased occurrence at the two extremes (i.e., small and large) of local permeability (Fig. 1A). In contrast, the DMM OA joints only showed increased occurrence at the lower end of local permeability (Fig. 1B). This result suggests that although the two OA models displayed similar mild OA-like symptoms, their calcified cartilage may have experienced different alterations during the process. Previous studies demonstrated more heterogeneous mineralization in aged human and monkey joints [7, 8]. We suspect that the same trend may have occurred in our ageing OA joints, while the DMM OA joints may have hyper-mineralized pockets in their calcified cartilage. We are mapping the matrix compositions in these OA joints to correlate with the functional permeability change. In summary, our study suggests that calcified cartilage in both normal and OA joints remains permeable to solutes, which may be involved in OA development.