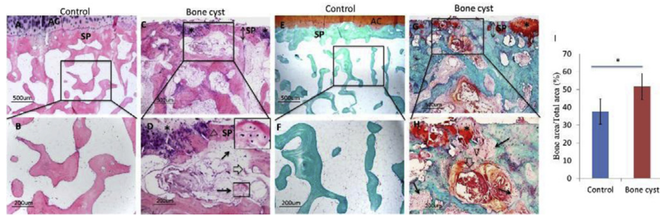

Besides, fibrocartilage tissue was found surrounding the bone cysts in bone cyst group. TRAP staining showed higher numbers of TRAP+ osteoclasts (61±7/mm2 vs. 36±4/mm2, p<0.05) in subchondral bone of the bone cyst group, indicating increased bone resorption. Higher numbers of Osteocalcin+ osteoblasts (58±5/mm2 vs. 35±4/mm2, p<0.05) and Osterix+ osteoprogenitors (65±6/mm2 vs. 37±4/mm2, p<0.05) were detected in areas surrounding bone cysts in the bone cyst group compared with the control group, indicating elevated level of osteoblastgenesis and bone formation.

Conclusions: These experiments demonstrate that subchondral bone cysts coincides with impaired subchondral bone microstructure in patients with knee OA. The underlying mechanism is possibly due to turnovers of subchondral bone remodeling.

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ALTERED NANO-STRUCTURAL AND NANO-MECHECANIAL PROPERTIES OF OSTEOARTHRITIS SUBCHONDRAL BONE

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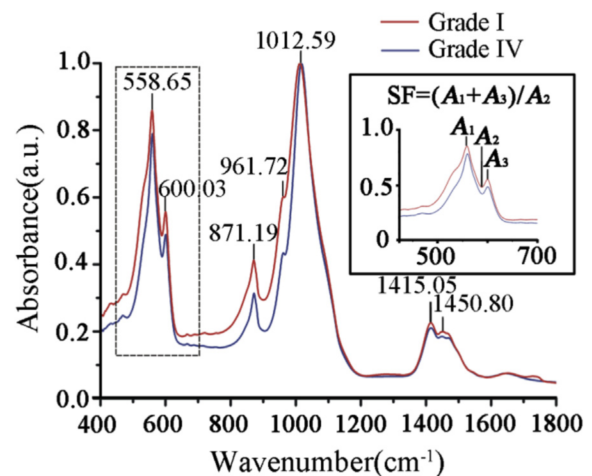
Purpose: The factors governing the altered strength of the subchondral bone in osteoarthritis (OA) are not well understood. In this study, we investigated how OA disease affects the subchondral bone structure and composition properties at a nano-scale level.

Methods: OA bone samples were collected from patients undergoing total knee replacement surgery and graded according to disease severity (grade I: mild OA; grade IV: severe OA). Transmission electron microscopy imaging, electron diffraction, and elemental analysis techniques were used to explore the type I collagen cross-banding pattern, the nature of mineral phase and the orientation of the crystal lattice. Furthermore, subchondral bone nanohydroxyapatite powders were prepared and characterised using X-ray diffraction, transmission electron microscopy, scanning electron microscopy and fourier transform infrared spectroscopy (FTIR). Subchondal bone bending strength and compressive strength were tested using a nano-indentation method.

Results: Grade I subchondral bone showed a clear apatite crystal profile with a typical cross-banding pattern in collagen fibrils. The c-axis orientation of the collagen fibrils was parallel to the long axis of fibrils in trabecular bone and the subchondral bone plate. However, in grade IV OA subchondral bone, the fibrils showed a random, undulated arrangement with circular oriented patterns of collagen fibrils in certain localized areas. Furthermore, the collagen fibrils showed abnormal intra-fibrillar mineralization and higher ratio of calcium (Ca) to phosphorous (P) (Ca/P) in grade IV OA bone samples. It was further observed that crystallinity of the mineral content in grade IV OA bone was greater than in grade I OA bone, which was confirmed by a higher splitting factor value (Figure 1). Nano-indentation revealed significantly reduced modulus and hardness in grade IV bone compared to the grade I bone (p<0.01), and which could lead to poorer mechanical strength of the grade IV bone.

Conclusions: These data suggest that severe OA affects the subchondral bone plate and trabecular bone by altering the nano-structural and mechanical characteristics, resulting in compromised mechanical properties of the subchondral bone.



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MULTICOLOR FLOW CYTOMETRY-BASED CELLULAR PHENOTYPING IDENTIFIES OSTEOPROGENITORS AND INFLAMMATORY CELLS IN THE OSTEOARTHRITIC SUBCHONDRAL BONE UNIT

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Purpose: Osteosclerosis of the subchondral bone due to aberrant bone remodeling is a pathological hallmark of osteoarthritis (OA). As the cellular component of the subchondral bone unit is thought to be responsible for the structural changes in this tissue, direct phenotypical analysis of the cellular compartment is critical to better understand the OA disease process. This study provides proof-of-principle that cells isolated from the subchondral bone unit can be directly phenotypically characterized without prior use of cell culture techniques.

Methods: Tibial plateaus were obtained from patients undergoing total knee arthroplasty. Subchondral bone chips (1 mm width, 1.5 g wet weight total) from nonsclerotic and sclerotic regions were digested in αMEM containing 0.6 mg/ml collagenase IA and hematopoietic and mesenchymal fractions of nucleated bone marrow cells were isolated using CD45 magnetic beads, followed by flow cytometry phenotpying. Mesenchymal cells were analyzed for expression of alkaline phosphatase (ALP) and osteocalcin (OC). Hematopoietic cells were phenotyped using multiple monocyte/macrophage markers (CD14, CD68, CD163, HLA-DR, CSFR). Presence of osteoblasts, macrophages and osteoclast progenitors was confirmed by (immuno)histochemical staining for OC, CD68 and tartrate-resistant acid phosphatase (TRAP), respectively.

Results: MTT staining revealed abundant viable cells and blood vessels in marrow cavities of subchondral bone chips pre-digestion. Collagenase digestion efficiently released fat tissue and marrow and bone-lining cells, evidenced by a strongly decreased MTT staining. Within the CD45-negative fraction the large majority of cells (>70%) expressed the mature osteoblast marker OC and approximately twenty percent of the cells were positive for the early osteoblast/osteoprogenitor ALP. The relative percentage of mature osteoblasts (CD45-/OC+) was slightly increased in sclerotic (64.2±10.3%) compared with nonsclerotic (50.5±12.5%) marrow tissues. Within the hematopoietic cell fraction, several distinct cell populations could be discriminated. The vast majority of cells were of monocytic origin (~80%) displaying strong surface expression of CD14 with or without co-expression of HLA-DR. In both nonsclerotic and sclerotic subchondral bone tissues, discreet macrophage populations (CD14+/HLA-DR+/CD68+) were identified and the percentage of macrophages was two-fold increased in the latter. Expression of the anti-inflammatory M2 macrophage marker CD163 in monocytic cells was very low (<2%). Putative osteoclast progenitors (CD45+/HLA-DR-/CD14-/CSFR+) were present in both subchondral bone phenotypes.

Conclusions: Flow cytometry analysis of the subchondral bone unit provides a powerful tool in helping to understand the cellular contribution to human OA. Osteoprogenitors/osteoblasts and inflammatory

cells reside in bone marrow cavities and their relative abundance changes between subchondral bone phenotypes. These data suggest that osteoimmunological interactions between these populations might be involved in OA bone remodeling.

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CHARACTERIZATION OF FOLATE RECEPTOR BETA EXPRESSION ON CELLS OF BONE ORIGIN

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Purpose: Despite being one of the most common types of arthritis and a leading cause of disability worldwide, effective therapeutics for osteoarthritis (OA) are still unavailable. We recently showed in a cross-sectional pilot study (25 patients, 50 knees) the localization of folate receptor beta (FR-β) positive macrophages in joints using 99mTc–EC20 imaging. The presence of activated macrophages, localized to the synovium, was associated with knee OA clinical symptoms; the presence of activated macrophages localized to the knee joint capsule of the tibiofemoral joint was associated with severity of knee joint space narrowing. However, 16% of the patients revealed positive 99mTc-EC20 uptake in the subchondral bone of osteoarthritic knees. The potential for expression of this receptor on cells of the bone has not been previously evaluated but is possible based on the knowledge that osteoclasts are multinucleated cells that derive from hematopoietic progenitors in the bone marrow, which also give rise to monocytes in peripheral blood, and to various types of tissue macrophages2. To better understand the cell types identified and targeted by folate conjugates and thereby assist with interpretation of 99mTc-EC20 images in patients with arthritis, the goal of this study was to evaluate the expression of folate receptors on cells of bone origin under resting and activated conditions.
Methods: The quantitation of FR-β, macrophage markers (CD14, CD163, CD64, CD80, TGF-B, and iNOS), osteoclast markers (cathepsin K and calcitonin receptor), and osteoblast markers (collagen type I and osteocalcin) mRNA was evaluated by reverse transcriptase polymerase chain reaction (RT-PCR) on human macrophage, osteoclast, and osteoblast cell lines. The expression of these proteins was also evaluated by multi-color confocal fluorescence microscopy using Alexa Fluor® 568, Alexa Fluor® 647, and fluorescein isothiocyanate conjugates.
Results: Gene expression analysis indicated the presence of low levels of FR-β in resting macrophages and precursor osteoclasts, and the absence of expression in osteoblasts, in comparison to the housekeeping genes ACTB and RPL37A. FR-β expression decreased 3.6 fold after osteoclast differentiation and maturation. Cell staining indicated similar results, suggesting that FR-β is primarily expressed on synovial macrophages.
Conclusions: These results suggest that synovial macrophages are the primary cell type expressing FR-β in the synovium of patients with OA. It is possible that targeting and inhibiting FR-β+ macrophages may be used to reduce the inflammation observed in OA.

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USING EPIPHYSEAL VOLUME TO SCREEN FOR GENES INFLUENCING OSTEOPHYTE FORMATION IN MUTAGENIZED MICE

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Purpose: Genetic modification in mice is a powerful tool for investigating pathways that influence the course of experimental osteoarthritis. For the most part these involve testing candidate pathways that have been identified from in vitro studies. The majority of published studies focus on the influence of such pathways on degeneration of the articular cartilage. Changes occurring in other tissues of the joint such as the bone, synovium and ligaments are relatively neglected. Mutagenesis programmes, in which random mutations are induced in populations of mice, provide an opportunity to discover novel genes in disease in an unbiased fashion. Here we report our preliminary data in which we use epiphyseal volume measurements, an indicator of osteophyte volume, after induction of OA, to screen for aberrant bone responses in mutagenized mice.
Methods: We are analyzing mice from the Harwell ENU mutagenesis programme, a large-scale phenotype-driven screen. Such screens make no a priori assumptions on the genetic basis of a phenotype and hence act as a discovery tool for the identification of new genes and pathways associated with a particular phenotype. Mice bearing random point mutations throughout their genome are analysed phenotypically and, once confirmed, outliers undergo SNP mapping and whole genome sequencing to identify the underlying mutation. In addition to the modifier screen, cohorts of the same mice are aged to 18 months as part of the Harwell Ageing screen. This provides the ability to screen the same mutations in induced as well as spontaneous (age-related) OA.
To date, 50 ten week old male mice from this on-going mutagenesis programme have undergone surgical destabilistion of the medial meniscus (DMM). At 4 weeks post surgery joints were scanned by microCT using CTan (2012-13 Bruker microCT) imaging software, and epiphyseal volume calculated by manually outlining the tibial epiphysis from the right (operated) and left (non-operated) knees (as described by us recently, Das Neves Borges et al, 2014). Selected joints were sent for serial sectioning and histological scoring to validate the bone phenotype and to look for associated joint changes in other tissues.
Results: We observed that at 4 weeks post DMM the majority of mice gained epiphyseal volume in the destabilized joint compared with the non-operated joint. By comparing the epiphyseal volumes in operated versus non-operated knees of individual mice, two pedigrees immediately stood out as outliers. One of these had higher non-operated epiphyseal volume but had an appropriate increase in volume upon joint destabilization. The second outlier had normal epiphyseal volume in the non-operated joint but lost substantial volume after joint destabilisation. 3 mice that were related to the latter pedigree showed a modest reduction in epiphyseal volume following DMM. Tissue samples from outlier mice have been sent for mapping and the lines have been re-derived for further in vivo studies.
Conclusions: Mutagenesis studies combined with rapid validated screening tests provide a realistic approach for discovering novel genes in OA pathogenesis.

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OSTEOSCLEROTIC BONE PHENOTYPE IS STABLY IMPRINTED IN SUBCHONDRAL MESENCHYMAL STROMAL CELLS IN HIP AND KNEE OSTEOARTHRITIS

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Purpose: The subchondral bone tissue plays a key role in the onset and progression of osteoarthritis (OA), since alterations of the bony bed precede cartilage lesions. Subchondral bone sclerosis in OA is characterized by an increase of bone material that is hypomineralized, caused by a dysregulated osteoblast phenotype. This increased bone anabolism suggests the involvement of mesenchymal stromal cell (MSC) recruitment and their differentiation into osteoblasts. In this study, we investigated whether the properties of these osteoprogenitors have changed in nonsclerotic and sclerotic OA subchondral bone.
Methods: Five knee tibial plateaus and four femoral heads were obtained from patients (mean age 65) undergoing total knee and hip arthroplasty, respectively. Subchondral bone tissues (± 1.5 g wet weight) from nonsclerotic and sclerotic regions were digested in αMEM containing 0.6 mg/ml collagenase IA for three hours and nucleated bone marrow cells were isolated by plastic adherence. First passage cells were seeded at colony forming densities and cultured for 2 weeks in control or osteogenic medium. Osteogenic (CFU-O) and total number of colonies (CFU-f) were counted after alkaline phosphatase (ALP) and methylene blue staining, respectively. Confluent cultures were subjected to osteogenic differentiation for three weeks and evaluated using ALP staining, quantitative ALP assays and qualitative analysis of mineralization by Alizarin Red staining.
Results: MSC clonogenicity, assessed by CFU-f counts, was 20.1±2.2% in nonsclerotic and 17.0±3.5% in the sclerotic subchondral tissue,