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±3/mm² vs. 35±4/mm², p<0.05) and Osterix+ osteoprogenitors (63±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 osteoblastogenesis 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.

**178 ALTERED NANO-STRUCTURAL AND NANO-MECHANICAL 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 subchondal 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-band 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-bandning 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.

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


**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 2.5% trypsin and the cell suspension was isolated using CD45 magnetic beads, followed by flow cytometry. Mesenchymal cells were analyzed for expression of alkaline phosphate (ALP) and osteocalcin (OC). Hematopoietic cells were phenotyped using multiple monocyte/macrophage markers (CD14, CD68, CD163, CD146, HLA-DR, C5). 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 post-digestion. Collagenase digestion efficiently released fat tissue and marrow and bone-lining cells, evidenced by a strongly decreased MTT staining. Within the CD45− 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, discrete 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 monocyctic cells was very low (<2%). Putative osteoclast progenitors (CD45+ HLA-DR+CD14−/C5+) 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...