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# Bone turnover and articular cartilage differences localized to subchondral cysts in knees with advanced osteoarthritis

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SUMMARY

*Objective:* To investigate changes in bone structure, turnover, and articular cartilage localized in subchondral bone cyst (SBC) regions associated with knee osteoarthritis (OA).
*Methods:* Tibial plateaus ($n = 97$) were collected from knee OA patients during total knee arthroplasty (TKA). SBCs were identified using micro-computed tomography, and the specimens were divided into non-cyst ($n = 25$) and bone cyst ($n = 72$) groups. Microstructure of subchondral bone was assessed using bone volume fraction (BV/TV), trabecular number (Tb.N), structure model index (SMI) and bone mineral density (BMD). In bone cyst group, the cyst subregion, which contained at least one cyst, and the peri-cyst subregion, which contained no cysts, were further selected for microstructure analysis. Articular cartilage damage was estimated using the Osteoarthritis Research Society International (OARSI) score. The numbers of $TRAP^+$ osteoclasts, $Osterix^+$ osteoprogenitors, $Osteocalcin^+$ osteoblasts and expression of SOX9 were evaluated by immunohistochemistry.
*Results:* Bone cyst group presented higher BV/TV, Tb.N and SMI at subchondral bone than non-cyst group. Furthermore, cyst subregion displayed increased BV/TV and Tb.N but lower BMD and SMI than peri-cyst subregion. Histology revealed a higher OARSI score in bone cyst group. SBC exhibited a weak relationship with BV/TV, *etc*. The numbers of $TRAP^+$ osteoclasts, $Osterix^+$ osteoprogenitors, $Osteocalcin^+$ osteoblasts and expression of SOX9, were higher in bone cyst group.
*Conclusion:* SBCs within knee OA are characterized by focally increased bone turnover, altered bone structure and more severe articular cartilage damage. The increased bone turnover possibly contributes to altered bone structure localized in SBC areas, and thus aggravates articular cartilage degeneration.


## Introduction

Osteoarthritis (OA) is one of the most prevalent joint disorders characterized by articular cartilage attrition and joint pain. Nowadays, OA is considered a disease of "the whole joint"[1,2]. The integrity and function of the cartilage can be influenced by pathological changes in structure and function of other joint tissues. Focal interaction among subchondral bone, marrow and cartilage in OA pathogenesis is drawing increasing attention[3–6].

Subchondral bone cysts (SBCs) were first defined as a concentric arrangement of trabeculae around an enlarged marrow space on radiographs[7,8]. On magnetic resonance imaging (MRI), SBCs demonstrate well-defined rounded areas of fluid-like signal intensity[9,10]. This allows the detection of small SBCs, and thus MRI is more sensitive than radiography in detecting SBCs[9]. Through the use of MRI, it has been reported that SBCs are present in up to 57% of OA patients[11]. The presence of SBCs is associated with knee

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pain[12], joint disability[13], cartilage loss, and increased risk of knee replacement in OA[14]. Two theories were supposed to explain the formation of SBCs: the "fluid breach theory"[15], and the "bony contusion theory" which claimed that, in the region of subchondral bone where the overloading exceeds the limits of physiological endurance, the bone dies and is liquefied[16,17]. SBCs were reported to develop in bone marrow lesions (BMLs) in patients with or at risk for knee OA, which supports the bony contusion theory[10]. Using micro computed tomography (micro-CT), studies revealed that trabeculae around SBCs was increased in density but decreased in mineralization than trabeculae unaffected by SBCs in human hip OA[15,16]. However, such changes have not been fully elucidated in human knee OA.

Histologically, necrotic bone fragments in SBCs and fibrous tissue surrounding cyst cavities were found in human OA knee[18]. Furthermore, immunohistochemical staining showed high expression of markers for osteoblast and osteoclast in SBCs in an animal model of secondary knee OA[19]. These studies suggest that changes in bone structure and remodeling occur in SBC regions. However, histopathology evaluation of cartilage and bone remodeling in SBCs within human knee OA are still poorly understood. Investigation of associations among bone remodeling and structure and cartilage in SBC regions will contribute to knowledge of OA pathogenesis.

Thus, we aimed to determine the changes in bone structure and turnover, and cartilage, within the SBC regions. We intended to identify the possible association between SBC formation and cartilage degeneration in the same subregions. By determining the contents within and surrounding SBCs, we hoped to provide insight into the origin of OA SBCs. We hypothesized that SBC regions in human OA knees are foci of increased bone remodeling and altered structure, and are spatially associated with articular cartilage degradation.

## Patients and methods

### *Subjects*

This study was approved by the Institution Review Board of the University of Hong Kong (Ref Nr: UW-09368). All patients gave informed written consent prior to their participation in this study.

A consecutive series of 97 patients with primary knee OA was enrolled in this study at the authors' hospital from February 2012 to April 2013. The diagnosis of OA was according to the classification of the American College of Rheumatology[20]. Inclusion criteria were Chinese living in Hong Kong aged between 45 and 75 years old. Patients were excluded if they had a history of knee joint trauma, other forms of arthritis, metabolic bone diseases, bone tumors, or were on any medications affecting bone remodeling. The patients' demographic data, including age, sex, and body mass index (BMI), were recorded. The large SBCs were observed using plain radiographs. The radiographic severity of OA were evaluated according to Kellgren and Lawrence (K–L) system[21] by an experienced reader (FL), using radiographs (standing anteroposterior view in 15° of flexion and a supine lateral view in 45° of flexion)[22] obtained 1 week prior to operation. Mechanical alignment of the lower extremity (the hip-knee-ankle angle) was assessed with full-limb standing radiographs (FL)[23]. The radiograph reader was blinded to patients' other information while performing K–L grading and assessment of mechanical alignment.

### *Micro-CT*

Tibial plateaus removed from the 97 patients during total knee arthroplasty (TKA) were collected. Then, macroscopic examination of these specimens was performed. Serial micro-CT scans of these 97 tibial specimens were carried out under a micro-CT scanner (Bruker micro-CT 1076, Belgium) with protocols described previously[24]. For three dimensional (3-D) measurements, the volume of interest (VOI) was selected as $10 \times 10 \times 5$ mm$^3$ of trabecular bone beneath the subchondral plate (SP) at the center of the medial tibial plateau (Supplementary Fig. 2A–D). The extraction of subchondral bone was performed with the aid of the edge detection function of MAT-LAB R2010a as previously described[24]. Briefly, the 2-D images were converted into discrete binary objects by the global thresholding and binarisation procedures using the software CTAn (Bruker micro-CT, Belgium). The irregular boundary of binary objects was detected after segmentation. The edges of bone cyst were saved as the region of interest (ROI) in the binary bitmap images, and unwanted edges were removed based on their coordinates in the segmented images to obtain the ROI for analysis.

The presence of SBCs was examined in all three anatomical planes using the software DataViewer (Bruker micro-CT, Belgium) (Supplementary Fig. 1). A bone cyst was defined as a spherical or ellipsoidal space that contained hardly any bone[15,16]. Only SBCs with a diameter greater than 1 mm were extracted, using an automatic method proposed by Chiba and colleagues[25,26] with the "morphology-based operations" model of the software CTAn. Briefly, trabeculae were dilated by 0.5 mm from the surface three-dimensionally, so bone marrow spaces less than 1 mm in diameter were closed. Next, the remaining spaces were dilated by 0.5 mm from the surfaces to restore their original shape. Then, after confirming the shape of the spaces (spherical or ellipsoidal), the SBCs were obtained[25,26]. Then the volume of SBCs was analyzed with the software CTAn. According to the absence or presence of SBCs, the subjects were divided into two groups: a non-cyst group ($n = 25$) and a bone cyst group ($n = 72$).

We further selected three subregions from the two selected VOIs in each of the two groups (Fig. 2a). Each subregion contained cylindrical subchondral bone of 3 mm in diameter and 10 mm in height. The three subregions were the "cyst" subregion, which contained at least one SBC ($n = 23$); the peri-cyst subregion, which was 3 mm adjacent to the cyst subregion in the same specimen and contained no bone cyst ($n = 23$); and the matched subregion, which is consistent with the anatomic location of cyst subregion ($n = 25$). If a peri-cyst subregion was not able to be selected, the specimen was excluded.

Then three dimensional (3-D) analysis of subchondral bone was performed using the software CTAn. The following parameters were calculated: bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), trabecular pattern factor (Tb.Pf)[27], structure model index (SMI)[28], degree of anisotropy (DA) and connectivity density (Conn.D). Moreover, the bone mineral density (BMD) was calibrated by using the attenuation coefficient of two hydroxyapatite phantoms (supplied by Bruker micro-CT, Belgium) with defined BMD of 0.25 g/cm$^3$ and 0.75 g/cm$^3$. The BMD color maps were established using the software CTvox (Bruker micro-CT, Belgium).

### *Histology*

After the micro-CT scan, tissue plugs corresponding to the VOIs of tibial plateaus ($n = 15$; samples were randomly selected from each group) were further processed for histological analysis. Serial sections were made into 5-μm thicknesses and stained with hematoxylin and eosin as well as Safranin O and Fast Green. Cartilage and subchondral bone regions were observed under light microscopy (Olympus DP 80). OA cartilage pathology was evaluated using the Osteoarthritis Research Society International (OARSI) scoring method[29]. Evaluations were conducted by an experienced expert (TW) blinded to the findings from radiographs, micro-CT and medical records. The ratio of bone area to total area of subchondral

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