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# Is subchondral bone cyst formation in non-load-bearing region of osteoarthritic knee a vascular problem?

Pok Man Boris Chan (BSc)[1#], Chunyi Wen (PhD)[2#*], Wai Ching Yang (BSc)[1], Chunhoi Yan (MBBS)[1], KwongYuen Chiu (MBBS)[1*]

[1]. Department of Orthopaedics & Traumatology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam

[2]. Interdisciplinary Division of Biomedical Engineering, Faculty of Engineering, The Hong Kong Polytechnic University, Hung Hom, Hong Kong

#: Equal contribution

*: Correspondence

## Introduction

Subchondral bone cyst (SBC), also known as "pseudo-cyst" and "geode" to orthopaedic surgeons, is one of textbook radiological features in osteoarthritis (OA) of the knee and shoulder. Previous reports stated SBCs commonly occurred underneath the joint surface subject to major mechanical loading where the articular cartilage were severly damaged. Given the evidence that the presence of SBCs is associated with greater loss of articular cartilage and increased risk of joint replacement surgery [1], it is obvious that the underlying pathomechanism of SBCs warrants further studies.

Two conflicting theories were proposed to address the aetiology of SBCs. One is the synovial intrusion theory – according to which synovial fluid is believed to be pumped into subchondral trabecular bone through microcracks on worn articular cartilage and damaged subchondral bone plate. This theory is widely accepted by orthopaedic surgeons and to a large extent veterinarians, as lollipop-shape synovial invaginations are sometimes observed in navicular bone of horses[2]. Another postulation is the bony contusion theory – in which bone bruising upon repetitive mechanical loading of subchondral trabecular bone is believed to be a prerequisite, and subsequent bone marrow oedema that fail to resolve due to chronic injuries and inflammation would further progress into SBCs. Whilst these theories may explain occurence of SBCs in load-bearing-regions caused by repetitive load-bearing and mechanical insults, both of them cannot explain the entire spectrum of SBC pathophysiology in OA. From our observations, SBCs do exist in non-load-bearing regions, like the tibial eminence and lateral compartment without apparent damage on overlying cartilage or fractures of the subchondral bone plate in addition to the usually reported load-bearing area with overlying cartilage defects (figure 1) [3], and in our previous cross-sectional study which analysed 144 tibial plateau specimens, for every 10 SBC identified, 5 were found in the tibial eminence and 1 would be in the lateral compartment. Some researchers also found SBCs in the tibial eminence [4] and lateral compartment[1, 5, 6] without visible overlying cartilage defects. Since articular cartilage has poor regeneration capacity, it is very unlikely that the surface heals itself post SBC formation, and whilst the lateral compartment SBCs may still be accounted for by the bony contusion theory to a certain extent since the compartment takes approximately 30% of the total load of the knee, SBCs present in the tibial eminence cannot be easily explained by this hypothesis, rendering these biomechanics-based theories far less sound and applicable. Apart from mechanical factors, excessive bone

resorption in rheumatic patients is a plausible contributing factor to the development of giant geodes in the subchondral bone. Moore et al. demonstrated that SBCs can originate as an isolated structure beneath the subchondral bone yet communicate with the synovial joint as it progresses based on a MRI-based case study of a RA patient[7]. Although RA is out of the scope of our study, this information does illustrate SBC formation could be a lot more complicated than otherwise thought.

To solve the mysterious occurrence of SBC in non-load-bearing region, our research group looked into systemic and local factors related to SBCs formation in knee OA and found hypertension, rather than obesity or weight, is a risk factor for SBC formation in these regions of the tibial plateau. However, this alone is insufficient in explaining the aetiology. Hence we would like to look deeper into how vascular pathology may lead to SBC formation.

## Hypothesis

Currently, neither the synovial intrusion theory nor the bony contusion theory can provide satisfactory explanation for the occurrence of SBC in the non-load-bearing region. We believe other non-mechanical factors may cause the development of SBC in the non-load-bearing region. The subchondral bone is a highly vascularised tissue, any disruption to the blood supply into the subchondral bone may cause detrimental consequences to the structure, one of them being bone resorption, which is a necessary step of bone geode formation. Angiotensin, which is the main vasoconstrictor and a major target for anti-hypertensive treatments, was found to be released by endothelial cells and act on osteoclasts thus leading to bone resorption[8]. In addition, it is known that bone marrow oedema (BMO) could be caused by reperfusion injury and that BMO can progress into SBCs[9], and given that our previous findings suggested a link between essential hypertension and SBC presence in the non-load-bearing region (table 1), *we hypothesise SBCs in the non-load-bearing region are primarily caused by ischaemic episodes of insufficient vascular supply in the subchondral bone area as a result of vascular ageing and endothelial dysfunction.*

To test our hypothesis, we must first proof the occurrence of vascular ageing. Apart from the altered vessel structure and relaxation response to nitric-oxide and enhanced endothelin-mediated contraction, vascular ageing and endothelial dysfunction have been characterised by reduced endothelial cell senescence, endothelial migration and proliferation, thus compromised angiogenesis for repairing[10]. This inability in tissue repairing results in chronic ischaemia of the tissue, compromised neo-angiogenesis and plausibly depletion of perivascular cells (pericytes) in the injured site compared to the case of normal vasculature. Three separate sets of experiments are listed below to confirm tissue ischaemia and vascular ageing of the subchondral bone.

Firstly, to demonstrate previous ischaemic episodes, immunohistochemical staining of hypoxic markers regulated by HIF-1 and with longer half-life like Glut-1 and PAI-1 [11] should be appropriate. Whilst the gold standard for demonstrating vascular ageing would be functional analysis of vascular response upon addition of relaxation and contraction factors, it is not feasible to harvest intact vessels from the subchondral bone. To demonstrate signs of endothelial senescence, histochemical staining of senescence-associated-β-galactosidase (SA-β-gal) would be required.

Secondary, vascular supply has been known to be a vital and indispensable component in osteogenesis ever since the discovery of roles of VEGF as a growth factor essential for bone development in addition to vessel ingrowth. Kusumbe *et al*. first classified endosteal vessels into L-type ($CD31^{lo}$, $EMCN^{lo}$) and H-type ($CD31^{hi}$, $EMCN^{hi}$) vessels and found H-type vessels, despite being present in low percentage, are responsible for angiogenesis and modulating osteoblastic cells[12]. These findings have been successfully replicated by other authors and were first validated in the context of OA progression by Cui *et al*.[13], in which excessive H-type vessel ingrowth into the subchondral bone is found to cause osteosclerosis. A recent report

also demonstrated that H-type vessels indeed play a role in maintaining bone mass in humans, and the depletion in such vessels results in bone loss, causing osteopenia [14]. We hypothesise insufficient H-type vessel ingrowth results in formation of SBC. This can be evaluated by immunofluorescent staining of CD31 & EMCN and subsequent histomorphometric analysis used in Wang and colleagues' study[14].

Pericytes around vessels is a source of potent mesenchymal stem cells and possibly osteoprogenitors[15], a cut-off could lead to osteopenia if not void formation. Rather than resulting in total collapse of subchondral bone in the case of "spontaneous osteonecrosis of the knee" (SPONK), the subchondral bone is temporarily stabilized by the formation of thickened trabeculae around the SBC to bear the load. To explore this hypothesis, SBCs and bone chips can be harvested from patients during total knee arthroplasty or subchondralplasty surgery and the cells can be harvested for flow cytometry analyses. Endothelial markers, mesenchymal markers, osteoprogenitor markers can be employed for general profiling of cell populations. Western blot and proteome can be used for testing the protein expression levels. A comparison study utilising transwell co-culture of normal pericytes with endothelial cells from the SBC explant against co-culture of normal pericytes with normal endothelial cells may be useful in investigating roles of defective endothelial cells in SBC formation. Our pilot qPCR study of SBC cell explants has shown significantly lower pericyte marker (MCAM) & osteoblast marker (OCN) levels and high MMP1 expression level in the SBC compared to non-sclerotic OA osteoblasts (figure 1B), in line with our hypothesis that pericyte and osteoprogenitors are depleted in SBCs.

*Through demonstrating occurrence of previous ischaemic episodes, endothelial senescence, insufficient H-type vessel ingrowth and loss of pericyte and osteoprogenitors in OA patients with SBC in the non-load-bearing regions, our hypothesis that vascular dysfunction may cause SBC in non-load-bearing region of the joint can be validated.*

**Discussion**

Currently, there is insufficient understanding for aetiology of SBCs in OA knees. Whilst previous theories may be able to explain formation of SBCs caused by overloading of the knee, the formation of SBCs in non-load-bearing region is another story and attempt to study them is like venturing in totally unknown waters due to lack of previous reports. In our recent cross-sectional study, we found hypertension to be a possible predictor of SBC formation in non-load-bearing region, which leads us to think about vascular pathology in SBC formation.

The linkage between vascular pathology and bone homeostasis has long been studied - vascular dysfunction is a proven risk factor of osteoporosis[16, 17]. Given both osteoporosis and SBC formation involves excessive bone resorption and insufficient repairing, we believe the molecular mechanisms behind could be similar if not directly translatable from one to another. But since previous correlational

studies show inverse relationship between osteoporosis and OA[18], it would seem counterintuitive to look into relationships between vascular dysfunction and OA. More recent evidence however proves this assumption to be wrong. Findlay's review[9] provided insights in how vascular pathology may contribute to OA pathogenesis and progression. We think the molecular mechanism behind osteoporosis may even be applicable in SBC

The idea that ischaemia may contribute in OA pathogenesis is not an entirely novel idea[19, 20]. Studies on hip joints in the past two decades revealed a distinct form of fracture of the subchondral bone termed "subchondral insufficiency fracture", which is different from osteonecrosis in a way that no sign of osteonecrosis can be seen prior to the collapse. This insufficiency fracture is caused by a normal or physiological stress loading to bone with suboptimal elastic resistance which tend to be osteopenic or osteoporotic[21]. However, the majority of past knee joint studies focused on ischaemia-induced osteonecrosis as a precursor of secondary OA rather than osteopenic bone due to chronic low-grade ischaemia as a primary cause of OA, or in our hypothesis a cardinal OA feature. There could be a blurred fine line between SPONK and subchondral insufficiency, a term coined by Bangil et al. in 1996[22]. It is plausible that this precise condition would result in partial collapse of the subchondral bone and thus SBC formation, rather than total collapse of trabecular structure as seen in osteonecrotic fracture which precedes secondary OA.

It would be counterintuitive to debride SBCs in order to treat OA because they would always come back if the problem cannot be nipped in the bud. Demonstrating vascular diseases could be the root of non-load-bearing region SBCs however, may provide clinicians with the opportunity to prevent their development, if not OA progression, with a conservative treatment that is not only cost-effective but also safer and less unpleasant to the patient. Apart from its value as an imaging biomarker for OA, the sight of non-load-bearing region SBC may also be seen as an indicator that flags underlying vascular disorder. Maybe SBC is still a relevant radiological sign after all?

175 **Table 1** Comparison of systemic and organ-specific functions in knee osteoarthritis patients with or without subchondral bone cyst formation

| Parameters | | | OA patients without bone cyst | OA patients with bone cyst in non-load-bearing regions | *p* value |
|---|---|---|---|---|---|
| Demographic Data | Age (years old) | | 72±11 | 72±9 | .980 |
| | Gender | Male | 5 | 8 | .731 |
| | | Female | 11 | 26 | |
| | Height (Meter) | | 1.51±0.05 | 1.52±0.06 | .602 |
| | Body Weight (Kg) | | 63±12 | 63±9 | .824 |
| | Body Mass Index (kg/m2) | | 28±5 | 26±3 | .325 |
| Blood pressure | Systolic blood pressure (mmHg) | | 167±23 | 167±12 | .961 |
| | Diastolic blood pressure (mmHg) | | 72±11 | 72±9 | .731 |
| | Mean arterial pressure (mmHg) | | 112±14 | 113±9 | .719 |
| | **Pulse pressure (mmHg)** | | **56±20** | **69±13** | **.027** |
| | Heart Beat (/minute) | | 98±10 | 97±15 | .773 |
| Blood | Hemoglobin (g/dL) | | 12±1 | 12±2 | .943 |
| Kidney | Urea (2.9-8.0 mmol/L) | | 6±2 | 7±3 | .080 |
| | Creatinine (49-82 umol/L) | | 75±29 | 82±24 | .431 |
| Liver | Total protein (67-87 g/L) | | 75±6 | 75±5 | .863 |
| | Albumin (39-50 g/L) | | 41±3 | 42±3 | .375 |
| | Globulin (26-40 g/L) | | 34±6 | 33±6 | .444 |
| | Alkaline phosphatase (47-124 U/L) | | 81±29 | 71±21 | .244 |
| | Alanine transaminase (8-45 U/L) | | 28±21 | 24±15 | .617 |
| | Aspartate transaminase (15-37 U/L) | | 37±7 | 25±8 | .647 |
| Glucose (<7.8 mmol/L) | | | 6.3±1.1 | 7.8±2.9 | .087 |
| Electrolytes | **Calcium (2.24-2.63 mmol/L)** | | **2.25±0.09** | **2.35±0.10** | **.033** |
| | Phosphate (0.88-1.45 mmol/L) | | 1.13 ± 0.18 | 1.06 ±0.15 | .367 |

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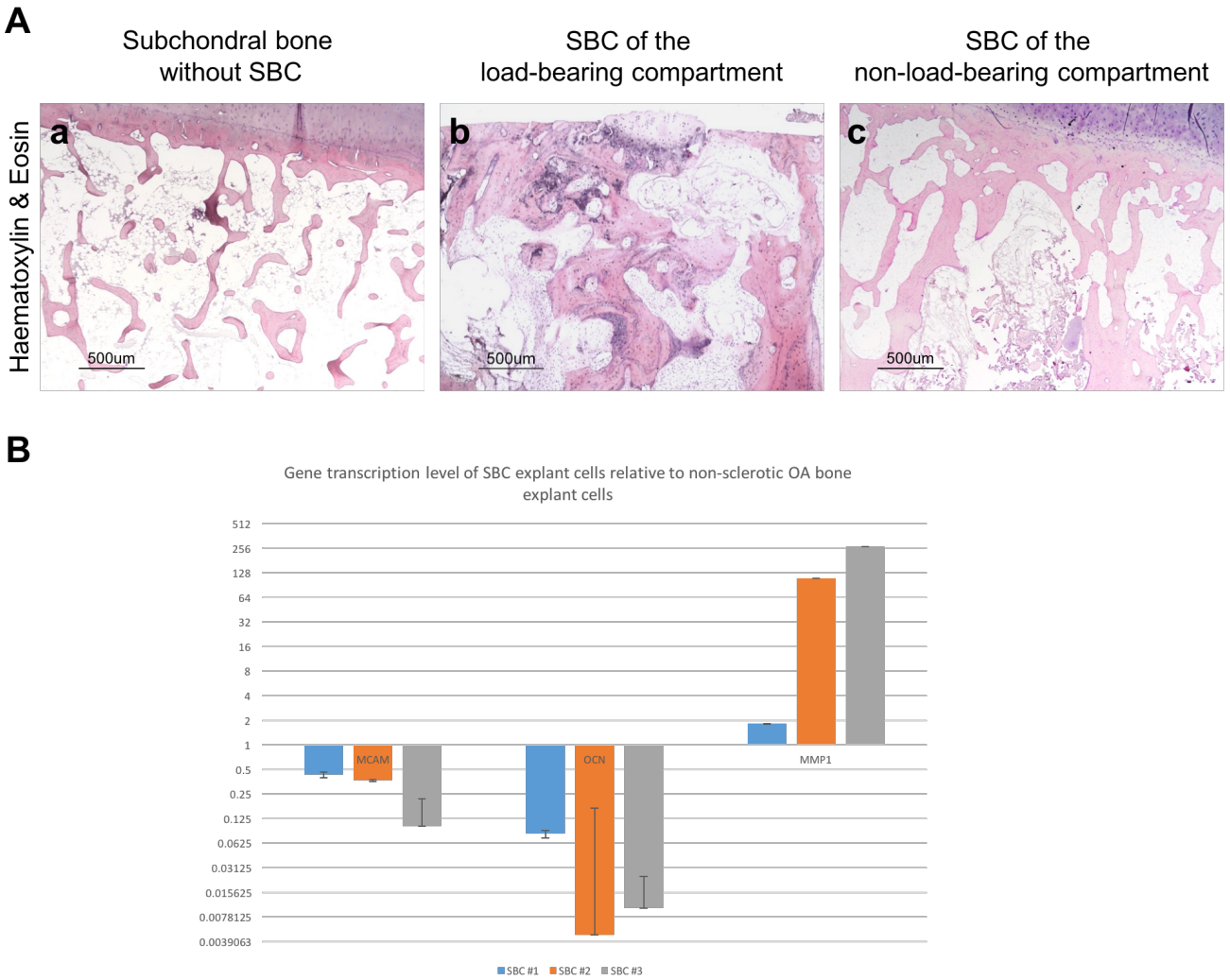


177 **Figure 1(A)** representative histological images of tibial plateau in advance knee osteoarthritis without subchondral bone cyst (a), with SBC of
178 the load-bearing compartment (b) and of the non-load-bearing compartment (c).
179 **1B** Relative Gene transcription levels of MCAM (CD146), Osteocalcin (OCN) and Matrix metalloprotease 1 (MMP1) of SBC explant cells.
180 Numbers are expressed in fold difference relative to non-sclerotic tibial plateau subchondral bone explant cells from the same patient.

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