
Article:
Li, Y., Liem, Y., Dall'Ara, E. orcid.org/0000-0003-1471-5077 et al. (4 more authors) (2021) Subchondral bone microarchitecture and mineral density in human osteoarthritis and osteoporosis : a regional and compartmental analysis. Journal of Orthopaedic Research, 39 (12). pp. 2568-2580. ISSN 0736-0266

https://doi.org/10.1002/jor.25018

Received: 4 November 2020 | Revised: 1 February 2021 | Accepted: 2 March 2021

DOI: 10.1002/jor.25018

RESEARCH ARTICLE

Journal of Orthopaedic Research®

Subchondral bone microarchitecture and mineral density in human osteoarthritis and osteoporosis: A regional and compartmental analysis

Yunfei Li[1] | Yulia Liem[1] | Enrico Dall'Ara[2] | Niall Sullivan[3] | Haroon Ahmed[4] | Ashley Blom[1,5] | Mohammed Sharif[1]

[1]Musculoskeletal Research Unit, Translational Health Sciences, Bristol Medical School, University of Bristol, Bristol, UK

[2]Department of Oncology and Metabolism and Insigneo Institute for in silico Medicine, University of Sheffield, Sheffield, UK

[3]Department of Trauma and Orthopaedics, Bristol Royal Infirmary, University Hospitals Bristol NHS Foundation Trust, Bristol, UK

[4]Bristol Medical School, University of Bristol, Bristol, UK

[5]National Institute for Health Research Bristol Biomedical Research Centre, University Hospitals Bristol NHS Foundation Trust and University of Bristol, Bristol, UK

Correspondence
Mohammed Sharif, Musculoskeletal Research Unit, Translational Health Sciences, Bristol Medical School, University of Bristol, Level 2, Learning and Research Bldg, Southmead Hospital, Bristol, BS10 5NB, UK.
Email: Mo.Sharif@bristol.ac.uk

Funding information
China Scholarship Council, Grant/Award Number: 201700260084

Abstract

Osteoarthritis (OA) and osteoporosis (OP) are historically considered to be inversely correlated but there may be an overlap between the pathophysiology of the two diseases. This study aimed to investigate the subchondral bone microarchitecture and matrix mineralization, and the association between them in OA and OP in relation to the degree of cartilage degeneration. Fifty-six osteochondral plugs were collected from 16 OA femoral heads. They were graded on a regional basis according to the stages of cartilage degeneration, as evaluated by a new macroscopic and a modified microscopic grading system. Twenty-one plugs were collected from seven femoral heads with OP. Plugs were scanned by microcomputed tomography and the microarchitectural and mineral properties were obtained for both subchondral plate and trabecular bone. Microarchitecture and material and apparent densities of subchondral bone in OP were similar to regions with early cartilage degeneration but different from regions with advanced cartilage degradation in OA femoral heads. Subchondral trabecular bone was more mineralized than subchondral plate in both OP and OA, and this compartmental difference varied by severity of cartilage degradation. Furthermore, the relationship among trabecular bone volume fraction, tissue mineral density, and apparent bone density was similar in OP and different stages of OA. Subchondral bone microarchitecture and mineral properties in OP are different from OA in a regionalized manner in relation to stages of cartilage degeneration. Both regional and compartmental differences at structural, material, and cellular levels need to be studied to understand the transition of OA subchondral bone from being osteoporotic to sclerotic.

KEYWORDS
cartilage degeneration, mineral density, osteoarthritis, osteoporosis, subchondral bone

1 | INTRODUCTION

Osteoarthritis (OA) and osteoporosis (OP) are two of the most common skeletal diseases in the ageing population.[1] The relationship between them has been a topic of discussion for decades. OA is characterized by cartilage degradation, subchondral bone sclerosis, synovial inflammation, and osteophyte formation at diarthrotic joints.[2] OP is characterized by systemic loss of bone due to unbalanced bone resorption, leading to an increased risk of fracture.[3]

Mechanism(s) of initiation and progression of OA remains unclear, but the interactions between cartilage and subchondral bone have been suggested to play a significant role.[4] In the 1970s, Radin et al.[5] hypothesized that sclerotic and stiff subchondral bone increases mechanical stress in cartilage and may be the cause of cartilage degradation. However, many studies reported that increased bone turnover and resorptive bone changes, rather than sclerosis, characterize early stage OA and/or promote disease progression.[2,6–9] Furthermore, the hardness of subchondral bone at tissue level has been reported to be reduced in OA compared with normal[10,11] and even with OP.[12] Such reduction has been attributed to increased bone remodeling and compromised matrix mineralization.[4,10,13–15]

Historically, OA and OP are thought to be inversely related as they rarely occur in the same patient,[16] and the presence of OA is generally associated with higher systemic bone mineral density.[1] One hypothesis is that more compliant subchondral support due to bone loss in OP protects the overlying cartilage.[14,15,17–19] However, the fact that increased remodeling and bone loss are also present in OA, as mentioned above, suggests a possible overlap between the pathophysiology of the two diseases.[20] This overlap provides the rationale for the use of bone-targeting agents as a potential disease modifying OA drugs (DMOADs).[20] But, various established anti-resorptive OP drugs, such as bisphosphonates and estrogen, have failed to show solid clinical evidence of efficacy for OA.[21]

These paradoxes point to a more complicated situation when studying the relationship between bone remodeling in OA and OP. First, OP is a systemic skeletal condition whereas bone changes in OA are mainly within the subchondral and adjacent epiphyseal/metaphyseal regions.[3,22] Bone remodeling in OA and OP need to be investigated in these areas rather than vertebral, iliac, or diaphyseal bone, to provide more relevant information. Second, subchondral bone properties are closely associated with the condition of the overlying cartilage in OA joint.[23–27] Such spatial variation is important as it may indicate the temporal change in OA development.[23,24,27,27,28] Third, microstructure and matrix mineralization of subchondral bone need to be studied simultaneously as the biomechanical properties of bone are determined at both apparent and material levels.[10,12,29] Moreover, the subchondral plate and trabecular bone compartments need to be studied concurrently as they are biologically and mechanically distinct and may have different impacts on the overlying cartilage.[9,30]

In our review of the scientific literature, we identified studies investigating one or more aspects of this broad topic.[17,24,31–33] They provided invaluable data contrasting subchondral bone remodeling in OP and OA, but none of them comprehensively addressed the relationship between OP and OA covering the whole criteria discussed above. Therefore, the purpose of this study was to investigate the differences in subchondral bone between OP and OA by comparing the microarchitecture, mineralization, and the correlation between microarchitecture and mineralization in a regionalized and compartmentalized manner, using a coupled macroscopic and microscopic sampling procedure.

2 | MATERIALS AND METHODS

2.1 | Patient selection

The study was approved by the Health Research Authority, UK (17/WS/0217). Written consent was obtained from patients before surgery at Southmead Hospital, Bristol, UK. Sixteen femoral heads were collected from patients (10 male and 6 female, mean age 68.1 ± 8.0 years) undergoing total hip arthroplasty (THA) for hip OA. Patients with known history of hip trauma, infection, avascular necrosis, and rheumatoid arthritis were excluded. Seven femoral heads were collected from patients undergoing THA for low-energy fracture of femoral neck, who were diagnosed with OP according to established clinical guidelines (4 male and 3 female, mean age 69.7 ± 5.9 years).[34,35] Patients with secondary OP due to prolonged use of corticosteroids, or on medications that affect bone metabolism were excluded. Inclusion criteria also involved a macroscopic inspection of specimens, as detailed below.

2.2 | Macroscopic evaluation and sampling

A macroscopic grading system, based on previously described methods[31,36,37] and our observations, was developed for this study to visually evaluate the severity of cartilage degeneration on the articular surface (Table 1 and Figure 1). For OA group, articular surface of each femoral head was divided and graded on a regional basis from Grade 1 to Grade 5. For the OP group, femoral heads from patients with hip fracture were included in the study only when their articular surface was normal or comparatively normal (Grade 1). After macroscopic evaluation, a steel hollow punch with 4 mm inner diameter was used to extract osteochondral plugs. OA plugs ($N = 56$) were collected from regions with different macroscopic Grades (one plug from each Grade) (Figure 1B–E). Macroscopic Grade 5 was not included in the study as the integrity of subchondral plate is compromised when bone is exposed.[38] Regions with bone cysts were also avoided. OP plugs ($N = 21$) were collected from three anatomical sites: anterior, posterior, and superior (Figure 1A). Plugs were kept frozen until micro-CT scanning. Before micro-CT scanning the macroscopic grade of each plug was re-evaluated to investigate intra-observer variability.

TABLE 1 The macroscopic grading system for cartilage degeneration used in this study

Macroscopic grade	Feature	Description
Grade 1	Normal or comparatively normal	White, or slightly gray; surface intact, smooth, no visible irregularity or fibrillation; elastic, no thinning or softening
Grade 2	Cartilage irregularity	Gray; surface intact, but with roughening, peeling and small fibrils; thinning, softening or swelling
Grade 3	Cartilage degeneration	Yellow, gray, or red; surface destructed, soft, fluffy, with severe fibrillation, cracks, and fissures; obvious thinning
Grade 4	Cartilage Erosion	Dark gray or yellow; surface can be smooth, roughened, or fluffy; cartilage almost worn off, with only a thin layer (<1 mm) left; immediately adjacent to exposed bone
Grade 5	Bone exposed	Cartilage completely worn off, with only subchondral bone left and exposed

2.3 | Microscopic evaluation and histology

After micro-CT scanning, plugs were fixed in formalin, decalcified, and embedded in paraffin. Osteochondral tissue sections with 7 μm thickness were cut. Four sections from each plug were randomly picked and stained with safranin O-fast green. The microscopic severity of cartilage degeneration was evaluated using the OARSI histopathology grading system,[36] with modifications to suit the localized sampling procedure (Table 2 and Figure 1). Two examiners, blinded to sample origins and macroscopic grades, scored sections independently. The scoring was carried out using the advanced grading with subgrade of 0.5 as shown in Table 2. Re-evaluation was accomplished with 8-week interval for intra-observer variability analysis. There were no samples with Grade 5–6.5 since regions showing complete loss of cartilage and osteophytes were excluded. Sections were viewed and photographed with DM5500 microscope and digital camera (Leica Microsystems).

2.4 | micro-CT scanning

micro-CT scanning was carried out with the Skyscan 1172 (Skyscan). Images were obtained with a 50 keV and 179 μA X-ray source. An isotropic voxel size of 4.87 μm was acquired, with 1180 ms integration time and 180° rotation. A 0.5 mm aluminum filter was chosen for reducing beam-hardening artifacts. Three-dimensional reconstruction was accomplished using NRecon (1.6.9.4; Skyscan). Reconstructed datasets were then imported into the CT Analyzer software (CTAn, 1.17.7.2; Skyscan) for processing and analysis.

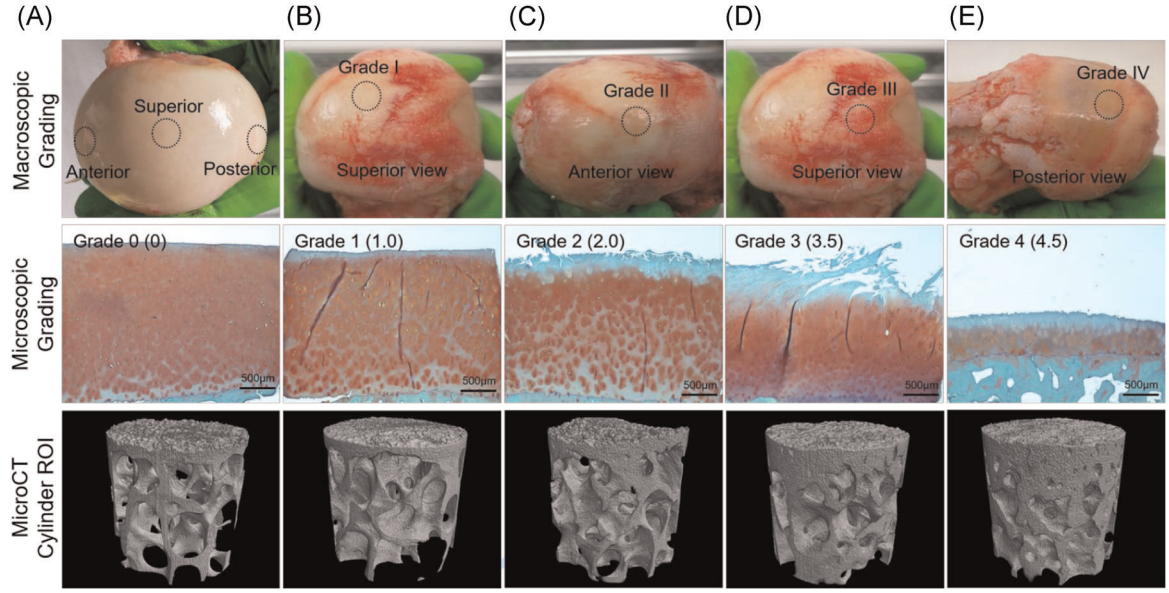

FIGURE 1 Macroscopic and microscopic evaluation of cartilage degeneration, and 3D cylindrical primary region of interest for micro-CT analysis. (A) Sample from a femoral head with osteoporosis. (B–E) Samples from the same femoral head with osteoarthritis (viewed from different angles). Circles: sites of osteochondral plug extraction. Microscopic grading is shown as generalized grading and advanced grading. Example 3D images were created by Amira (2020.1; Thermo Fisher Scientific) [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 The modified OARSI microscopic grading system used for evaluation of cartilage degeneration

		Matrix structure					Chondrocyte biology		
Generalized grading	Advanced grading	Surface	Fibrillation	Fissure	Matrix loss	Others	Proliferation and cluster (C)	Others	Proteoglycan depletion
0	0.0	Intact and smooth	N/A	N/A	N/A	N/A	(-)	N/A	SZ only
1	1.0	Intact but irregular	Superficial, usually appears as irregularity or small fibrils on the cartilage surface	N/A	N/A	Matrix hypertrophy or atrophy (B)	(-) to (+)	N/A	SZ only
	1.5	Intact but irregular	As above, with superficial microcracks	N/A	N/A		(-) to (+)	Cell death (empty lacunae, membrane 'ghost', fragmented nuclei), cell hypertrophy (increased size and chondron staining), disrupted alignment of cells.	SZ only
2	2.0	SZ(A) exposed	Discontinuity and/or microcracks into and confined to SZ	N/A	Superficial abrasion, floating matrix 'flakes', 'fibrils'	(+)	SZ to Upper 1/3 MZ		
	2.5	SZ exposed	Discontinuity and/or microcracks through SZ	N/A	As above, deeper and through SZ	(+)	Upper 1/3 MZ		
3	3.0	Upper 1/3 of MZ exposed	N/A	Simple, confined to upper 1/3 MZ	Deep spallation along the fissures, with major loss of SZ	N/A	(+) to (++)		Upper 1/3 MZ or lower
	3.5	Lower 2/3 of MZ exposed	N/A	Branched, or simple but reaches lower 2/3 MZ	Deep spallation along fissures, with complete loss of SZ		(++)		Lower 2/3 MZ to DZ
4	4.0	MZ-DZ junction exposed	N/A	Down to MZ-DZ junction	Erosion/excavation down to MZ-DZ junction, with partial loss of MZ		(++)		DZ
	4.5		N/A				(-) to (++) (C)		DZ

Generalized grading	Advanced grading	Matrix structure					Chondrocyte biology		Proteoglycan depletion
		Surface	Fibrillation	Fissure	Matrix loss	Others	Proliferation and cluster (C)	Others	
		DZ exposed, only a thin layer of cartilage attached to SB		Down to lower DZ. Or no fissure presents, just jigsaw-like surface	Erosion/ excavation down to lower DZ, with complete loss of MZ				
5	5.0	Subchondral bone exposed, with (5.5) or without(5) reparative tissue							
	5.5								
6	6.0	Marginal (6) or central (6.5) osteophyte observed							
	6.5								

Note: (A) Superficial zone (SZ), middle zone (MZ) and deep zone (DZ) are grossly divided according to the shape and alignment of chondrocytes, occupying approximately 10%, 60%, and 30% of cartilage full depth, respectively. (B) For small and localized sample, matrix hypertrophy or atrophy is difficult to identify on histology sections. Overall macroscopic inspection during the sampling procedure needs to be considered. (C) Chondrocyte cluster is defined by the number of cells in the same lacunae: (-) < 4; 4 ≤ (+) < 10; (++) ≥ 10. At Grade 4.5, clusters may not be observed due to cartilage loss.

For cartilage changes in OA, the generalized Grades 1 and 2 (advanced Grade 1–2.5) are considered as early degeneration; generalized Grades 3 and 4 (advanced Grades 3–4.5) are considered as advanced degeneration; generalized Grades 5 and 6 (advanced Grades 5–6.5) are considered as end-stage degeneration.

For detailed nomenclature of histologic descriptions, refer to the Appendix A in Pritzker et al.[36]

2.5 | Image processing

A global threshold was used for binarization to differentiate between bone and marrow. A cylindrical region of interest (ROI) with 3.0 mm diameter and 4.0 mm depth (Figure 1) was selected in the middle of plugs. This preliminary ROI was then segmented into ROIs of subchondral plate and trabecular bone using a semiautomated method (CTAn). Briefly, the "narrowing points" where trabeculae starts to stretch out from the bottom of subchondral plate were manually identified and lined up. The drawing was repeated on every 3–5 slices depending on the variation. The lines between slices were automatically interpolated. The data set above this line was further contoured by the Shrink-Wrap function (CTAn) to remove redundant image areas and marrow space, creating the subchondral plate ROI. The data set beneath this line constituted the trabecular ROI.

2.6 | Microarchitecture analysis

Microarchitecture of subchondral plate and trabecular bone were analyzed automatically within the corresponding ROIs (CTAn). For trabecular bone, bone volume fraction (bone volume/total volume, BV/TV), specific bone surface (bone surface/bone volume, BS/BV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), trabecular number (Tb.N), structural model index (SMI), and connectivity density (Conn.Dn) were measured as described previously.[39] For subchondral plate, plate thickness (Pl.Th) was calculated by applying the sphere-fitting method[39] to the contour of subchondral plate ROI. Total porosity (Pl.Po) was calculated as volume of pores per volume of the plate ROI.

2.7 | Mineral density analysis

Two calcium hydroxyapatite phantoms of known mineral density (0.25 and 0.75 g/cm^3) were scanned and reconstructed under the same conditions described above. The X-ray attenuation coefficient values of these phantoms were recorded and a linear calibration equation was used to calculate the mineral density of samples. The apparent density, or volumetric bone mineral density (BMD), was defined as mineral density over the total volume of the ROI, including bone and marrow space.[39] It was measured for trabecular bone only. The material density, or tissue mineral density (TMD), was measured as mineral density over the volume of bone only.[39] TMD reflects the mean degree of bone matrix mineralization[25,40] and was measured for both subchondral plate and trabecular bone. For measurement of TMD, the outer layer (1 voxel in thickness) was removed from the bone surface to correct for partial volume effect.

2.8 | Statistical analysis

Inter- and intra-observer variability were evaluated by intraclass correlation coefficient (ICC).[41] Correlation between macroscopic and

TABLE 3 Analysis of variance of microarchitecture and mineral densities in relation to the severity of cartilage degeneration in OA and OP

	OP (*N* = 21)	OA Grade 1 (*N* = 14)	OA Grade 2 (*N* = 12)	OA Grade 3 (*N* = 14)	OA Grade 4 (*N* = 16)	*p* value (OA grades only)	*p* value (OP and OA grades)
Tb.TMD (g/cm^3)	1.41 ± 0.06	1.45 ± 0.11	1.39 ± 0.07	1.27 ± 0.07	1.24 ± 0.09	<.001	<.001
Pl.TMD (g/cm^3)	1.22 ± 0.07	1.20 ± 0.09	1.17 ± 0.07	1.17 ± 0.07	1.15 ± 0.07	.300	.0322
BMD (g/cm^3)	0.27 ± 0.10	0.20 ± 0.14	0.31 ± 0.11	0.42 ± 0.10	0.44 ± 0.14	<.001	<.001
BV/TV (%)	25.64 ± 6.24	22.04 ± 8.81	27.32 ± 7.19	35.93 ± 7.95	39.06 ± 10	<.001	<.001
Tb.Th (mm)	0.18 ± 0.03	0.16 ± 0.03	0.17 ± 0.03	0.20 ± 0.03	0.22 ± 0.04	<.001	<.001
Tb.N (mm^{-1})	1.40 ± 0.27	1.37 ± 0.45	1.64 ± 0.41	1.75 ± 0.29	1.81 ± 0.28	.0089	<.001
Tb.Sp (mm)	0.55 ± 0.08	0.58 ± 0.13	0.50 ± 0.12	0.50 ± 0.10	0.46 ± 0.08	.052	.0235
SMI (-)[a]	1.31 ± 0.22	1.27 ± 0.42	1.13 ± 0.37	0.37 ± 0.79	0.05 ± 1.32	<.001	<.001
Conn.Dn (mm^{-3})[a]	2.25 ± 1.28	2.42 ± 1.33	3.73 ± 1.89	4.26 ± 1.90	5.93 ± 2.49	<.001	<.001
BS/BV (mm^{-1})	20.64 ± 3.46	23.34 ± 4.00	21.93 ± 3.23	17.97 ± 2.25	17.40 ± 3.41	<.001	<.001
Pl.Th (mm)	0.26 ± 0.04	0.29 ± 0.06	0.33 ± 0.06	0.39 ± 0.09	0.42 ± 0.11	<.001	<.001
Pl.Po (%)	9.75 ± 3.11	8.72 ± 2.20	8.57 ± 2.43	8.79 ± 2.32	8.41 ± 2.65	.9738	.554

Note: Values are mean ± *SD*. Analysis was first made between OA grades to examine the regional variations in relation to the severity of OA. Then the tests were repeated to include the OP group to compare OP with OA grades. One-way ANOVA was used for parametric data.

Abbreviations: ANOVA, analysis of variance; BMD, bone mineral density; BS/BV, bone surface/bone volume; BV/TV, bone volume/total volume; OA, osteoarthritis; OP, osteoporosis.

[a]Kruskal-Wallis test was used for non-parametric data.

microscopic grading was evaluated by Spearman's rank test.[41] Since we collected plugs based on the condition of the overlying cartilage and were interested in how cartilage degeneration was related to changes in subchondral bone, we assumed independence of samples in this study. Normal distribution of bone architectural and mineral parameters was inspected by Shapiro-Wilk test. Comparisons of these parameters were first made between microscopic grades within OA group to verify the regional variations in relation to the severity of cartilage degeneration, using one-way analysis of variance (ANOVA) (with Bonferroni correction) for parametric data or Kruskal-Wallis test (with Dunn's correction) for non-parametric data. These tests were repeated to include OP group and compare OP with different microscopic grades of OA. Comparisons of TMD between subchondral plate and trabecular bone (both parametric) were carried out by paired Student *T* test, matching the two compartments for each osteochondral plug. The association among BV/TV, TMD and BMD was investigated using linear regression. Slopes of regression lines were compared using the general linear model. Results were presented as mean and standard deviation (*SD*) unless otherwise indicated. Statistical significance was indicated by two-tailed *p* value less than .05. IBM SPSS (26.0, IBM Corp.) and GraphPad Prism (8.3.0, GraphPad Software) were used for statistical analysis and graphing.

3 | RESULTS

3.1 | Macroscopic and microscopic evaluation

Macroscopic grading was carried out by one observer (Li) and the intra-observer ICC was 0.934. Microscopic grading was carried out by two observers using the advanced grading with subgrade of 0.5 to evaluate the reliability of the modified OARSI grading system. The interobserver ICC for microscopic grading was 0.967 and the intra-observer ICCs were 0.974 (Li) and 0.972 (Liem), respectively. The macroscopic and microscopic grading systems showed an excellent correlation as indicated by the Spearman's coefficient of 0.924. The osteochondral plug sampling procedure covered the range of cartilage histopathological grades and was capable of differentiation between them (Figure S1).

In the following comparisons of bone microarchitecture and mineral properties, for simplicity and due to limited sample size, the advanced grading of OA plugs was generalized to Grades 1, 2, 3, and 4 as in Table 2. In addition, the advanced grading of OP plugs ranged from 0–1.5, and 1–1.5 were treated as acceptable age-related minor degeneration, accordingly the data from the OP group were pooled without further differentiation.

3.2 | Mineral densities

The TMD and results of analysis of variance are reported in Table 3. Results of post-hoc multi-comparisons are depicted in Figure 2A. The data show a significant reduction in the TMD of trabecular bone (Tb.TMD) with increasing severity of cartilage degradation in the OA group ($p < .001$; Table 3 and Figure 2A,C). Both Grades 1 and 2 had a value significantly higher than Grades 3 and 4 (Figure 2A). The Tb.TMD of the OP group showed no statistically significant difference compared to OA Grade 1 and 2 but was significantly higher than OA Grades 3 and 4 ($p < .001$ for both OP vs. OA Grades 3 and 4 (Figure 2A,C). The TMD of subchondral plate (Pl.TMD) in the OA samples showed no regional

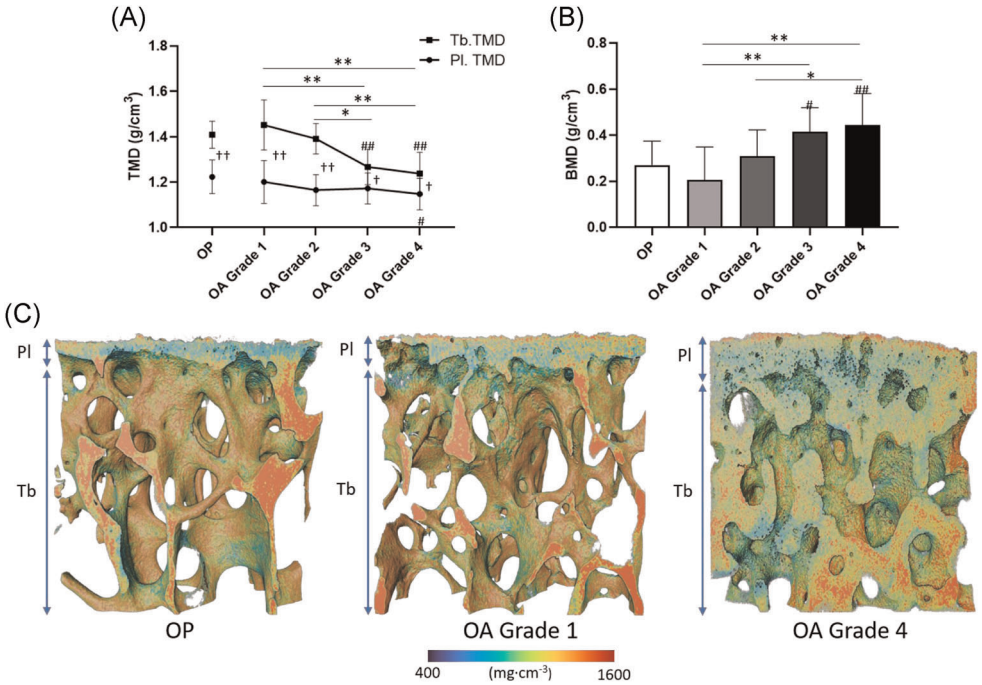

FIGURE 2 Comparisons of mineral density. (A) Comparisons of TMD in relation to microscopic grading of cartilage degeneration and between subchondral plate and trabecular bone. (B) Comparisons of BMD of trabecular bone in relation to microscopic grading of cartilage degeneration. (C) 3D cross-sections of subchondral bone colored to indicate level of mineralization, showing that trabeculae in OP and OA Grade 1 is more mineralized than in OA Grade 4, and mineralization is higher in trabecular bone than in subchondral plate. (C) also shows the porotic bone structure in OP and OA Grade 1, and sclerosis in OA Grade 4. Graphs show mean ± *SD*. * and # indicate the significance of comparisons between OA grades, and between OP and OA grades, respectively, using post-hoc Bonferroni test. †Indicates comparisons between subchondral plate and trabecular bone using Paired Student *T* test. *$p < .05$; **$p < .001$; the same for # and †. Example 3D images were created by Amira (2020.1, Thermo Fisher Scientific). BMD, bone mineral density; OA, osteoarthritis; OP, osteoporosis; Pl, subchondral plate; Tb, trabecular bone; TMD, tissue mineral density [Color figure can be viewed at wileyonlinelibrary.com]

differences related to microscopic grades ($p = .300$; Table 3 and Figure 2A), but the value for OA Grade 4 was significantly lower than that of OP (1.15 ± 0.07 g/cm^3 vs. 1.22 ± 0.07 g/cm^3, $p = .035$; Figure 2A). For compartmental comparisons, trabecular bone had a significantly higher TMD than subchondral plate in all study groups (Figure 2A,C). The difference was larger in OP, and OA Grades 1 and 2 (+15%, +21%, +19%, respectively, $p < .001$), and smaller in OA Grades 3 and 4 (+8%, +8%, $p = .005$ and .007, respectively).

The BMD of trabecular bone in OA increased with histopathological grading ($p < .001$; Table 3 and Figure 2B). OA Grades 3 and 4 had a value significantly higher than Grade 1 and/or Grade 2 (Figure 2B). The trabecular BMD of OP showed no statistically significant difference with OA Grades 1 and 2 but was lower than that of OA Grades 3 and 4 (Figure 2B).

3.3 | Microarchitecture

For microarchitecture of trabecular bone, overall, a significant regional variation in relation to cartilage degradation in OA was found, except for Tb.Sp. Specifically, BV/TV ($p < .001$), Tb.Th ($p < .001$), Tb.N ($p = .009$), and Conn.Dn ($p < .001$) increased significantly for higher OA grades, whereas SMI and BS/BV decreased significantly ($p < .001$) with the increasing OA Grades (Table 3 and Figure 3). When the OP group was included in the analysis, post hoc comparisons demonstrated that BV/TV, Tb.Th, Tb.N, and Conn.Dn of OP were significantly lower compared with those of OA Grade 3 and/or Grade 4, whereas SMI and BS/BV of OP were significantly higher than those of OA Grade 3 and/or Grade 4 (Figure 3). An exception was Tb.Sp which showed a downward trend with increased OA grades, but this was not statistically significant ($p = .052$. Table 3). Tb.Sp showed no significant difference between OP and OA grades (Figure 3).

Subchondral plate thickness (Pl.Th) in OA increased significantly with worsening cartilage histopathology ($p < .001$; Table 3 and Figure 3). The OP group had a significantly thinner plate compared to OA Grades 2, 3, and 4 ($p = .017$, $p < .001$, $p < .001$, respectively; Figure 3). No statistically significant differences were found between all study groups in terms of Pl.Po (Table 3 and Figure 3).

3.4 | Correlation between BV/TV and mineral density

The results of the linear regression analyses among BV/TV, TMD, and BMD are summarized in Table 4 and depicted in Figure 4. In both OP and OA group, BV/TV was positively associated with BMD (OP: $R^2 = 0.98$, $p < .001$; OA: $R^2 = 0.96$, $p < .001$) but was inversely associated with TMD (OP: $R^2 = 0.82$, $p < .001$; OA: $R^2 = 0.85$,

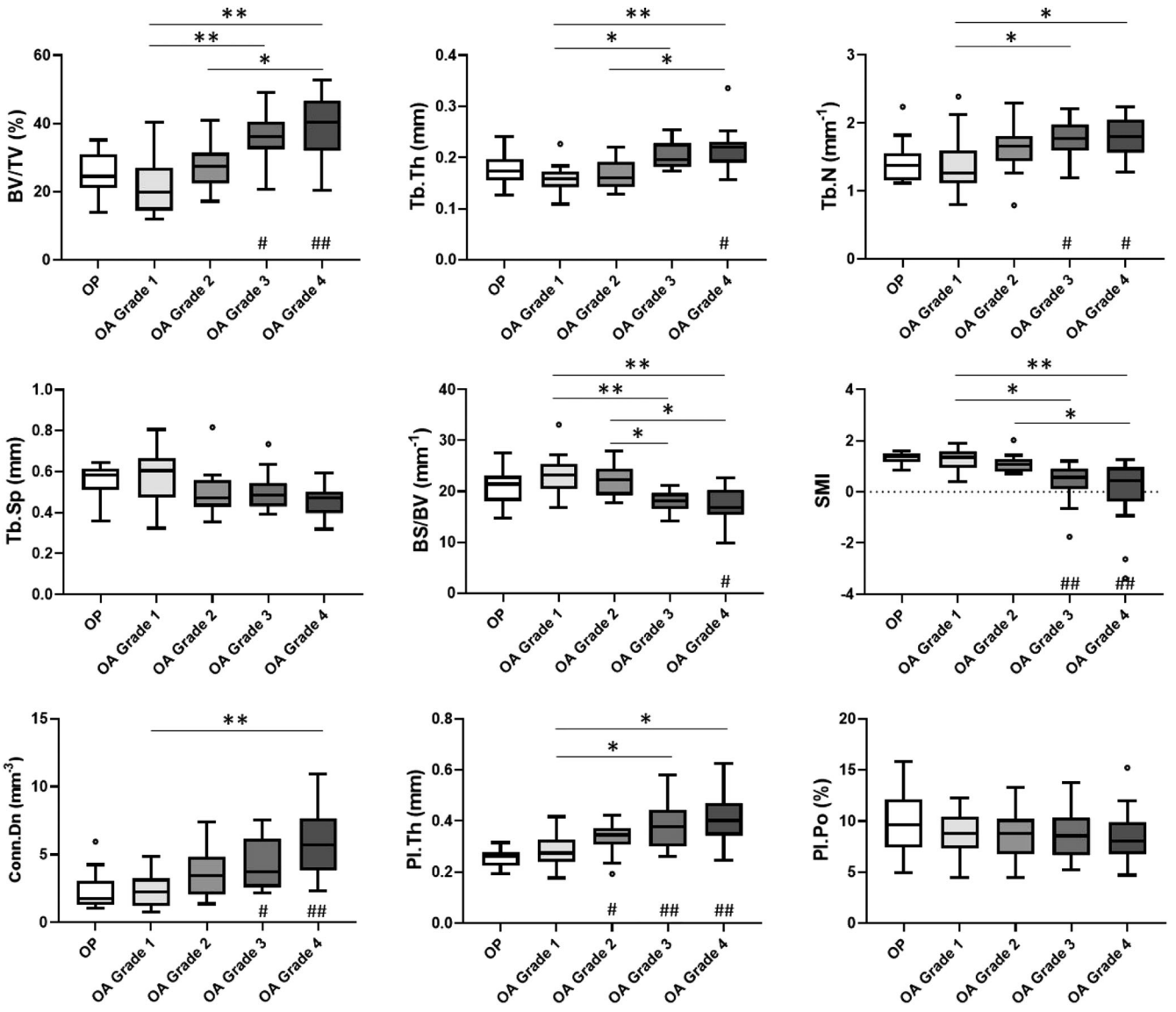

FIGURE 3 Comparisons of microarchitecture of subchondral plate and trabecular bone in relation to microscopic grading of cartilage degeneration. The boxplot shows the median, the interquartile range (IQR), the highest and lowest value within 1.5 IQR, and outliers. Statistical significance is indicated by * or # for multiple comparisons between osteoarthritis (OA) grades, and between osteoporosis (OP) and OA grades, respectively, using post-hoc tests. *$p < .05$; **$p < .001$; the same for #

$p < .001$). Furthermore, TMD was inversely correlated with BMD in both groups (OP: $R^2 = 0.81$, $p < .001$; OA: $R^2 = 0.81$, $p < .001$). The pattern of the above correlations remained the same when analyzed separately for each OA grade and there was no statistical difference in the slopes of regression lines between the OP and OA group, or between OA grades (Figure 4 and Table 4).

4 | DISCUSSION

In this study, we used a combined macroscopic and microscopic sampling procedure which permitted a better representation of the regional difference in subchondral bone microarchitecture and matrix mineralization in relation to the severity of cartilage degeneration. We showed that subchondral bone properties in OP was similar to regions with early but different from regions with advanced cartilage degeneration in OA. We also introduced, for the first time, a compartmental comparison of TMD between subchondral plate and trabecular bone, showing that trabecular bone is more mineralized than subchondral plate in OP and OA, and this compartmental difference varied with severity of cartilage degeneration. Our data also showed that the relationship among bone volume fraction, material density, and apparent density was similar in OP and in different stages of cartilage degradation in OA, which has not been reported previously.

A number of earlier studies using tissue samples from fixed site (s) regardless of the status of the overlying cartilage showed that the matrix mineralization of subchondral trabecular bone, measured by

TABLE 4 Linear regression between bone volume/total volume (BV/TV), material density (TMD), and apparent density (BMD) of trabecular bone in osteoporosis (OP) and osteoarthritis (OA) group, and in each grade of OA group

	BV/TV versus TMD				BV/TV versus BMD				TMD versus BMD			
	p value	R^2	Slope (95% CI)	Intercept (95% CI)	*p* value	R^2	Slope (95% CI)	Intercept (95% CI)	*p* value	R^2	Slope (95% CI)	Intercept (95% CI)
OP group (*N* = 21)	<.001	0.81	−0.009 (−0.011, −0.007)	1.630 (1.580–1.681)	<.001	0.98	0.016 (0.015, 0.018)	−0.148 (−0.180– −0.116)	<.001	0.81	−1.563 (−1.931, −1.195)	2.473 (1.955–2.992)
OA group (*N* = 58)	<.001	0.85	−0.011 (−0.012, −0.009)	1.664 (1.623–1.705)	<.001	0.96	0.014 (0.013, 0.015)	−0.093 (−0.120– −0.066)	<.001	0.81	−1.125 (−1.274, −0.976)	1.846 (1.647–2.045)
OA Grade 1 (*N* = 14)	<.001	0.84	−0.011 (−0.015, −0.008)	1.704 (1.630–1.779)	<.001	0.97	0.016 (0.014, 0.018)	−0.150 (−0.189– −0.111)	<.001	0.85	−1.204 (−1.523, −0.885)	1.953 (1.488–2.417)
OA Grade 2 (*N* = 12)	<.001	0.72	−0.008 (−0.011, −0.004)	1.608 (1.509–1.708)	<.001	0.95	0.015 (0.013, 0.018)	−0.111 (−0.183– −0.039)	<.001	0.78	−1.484 (−2.041, −0.928)	2.375 (1.599–3.150)
OA Grade 3 (*N* = 14)	<.001	0.65	−0.008 (−0.012, −0.004)	1.552 (1.419–1.685)	<.001	0.93	0.013 (0.010, 0.015)	−0.076 (−0.115– 0.043)	.003	0.53	−0.965 (−1.541, −0.389)	1.639 (0.908–2.370)
OA Grade 4 (*N* = 16)	<.001	0.76	−0.008 (−0.011, −0.006)	1.556 (1.451–1.661)	<.001	0.94	0.013 (0.011, 0.015)	−0.071 (−0.150– 0.008)	<.001	0.65	−1.180 (−1.674, −0.686)	1.905 (1.292–2.519)

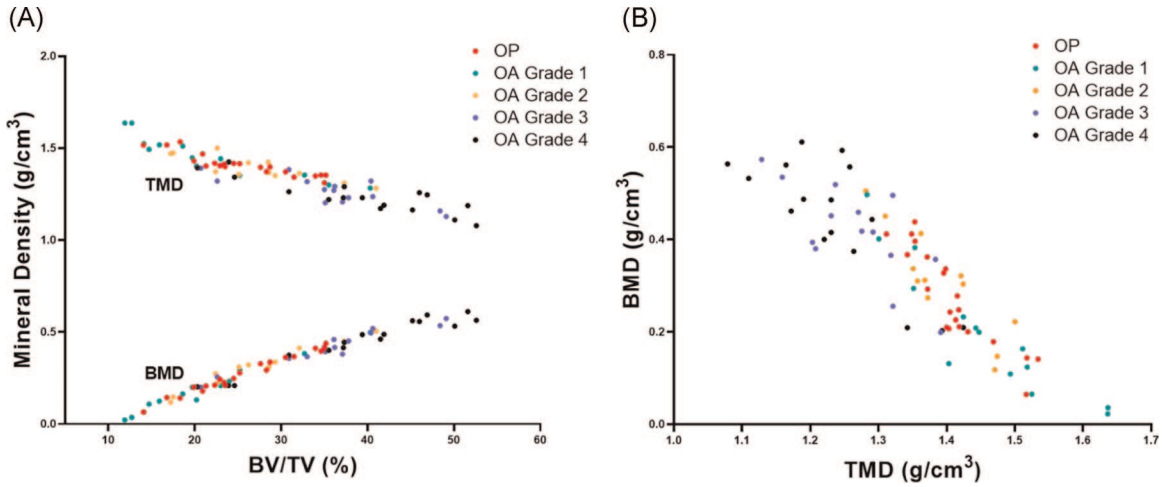

FIGURE 4 Correlations among bone volume/total volume (BV/TV), material density (TMD), and apparent density (BMD) of trabecular bone in osteoporosis (OP) and osteoarthritis (OA) group, and in each grade of OA group. (A) Correlations between BV/TV and TMD, and between BV/TV and BMD. (B) Correlations between BMD and TMD [Color figure can be viewed at wileyonlinelibrary.com]

material density or TMD, decreased in OA compared to healthy and OP.[13,15,17] More recently it was shown that trabecular bone was more hypo-mineralized in regions with complete cartilage loss compared to those still covered by cartilage in OA specimens.[24,25] Our results are consistent with these studies and additionally showed that the changes were stagewise in terms of cartilage degeneration and already significant before exposure of subchondral bone, thus providing further evidence that subchondral bone mineralization and cartilage degradation are intrinsically related in OA. The matrix mineralization of trabeculae in OP was shown to be decreased,[42] increased,[43] or unchanged[15] compared to control by different studies. The conflicting results are likely to be due to site of tissue sampling (spine, ilium, femur, etc.) and the stage of disease.[44,45] In this study, we focused on the subchondral bone of hip joint in established OP and OA and showed that trabecular mineralization of OP was similar with that of regions with early cartilage degradation in OA, and significantly higher than that of regions with advanced degradation. This cartilage degradation based regionalized observation may partly explain why the tissue hardness of trabeculae, measured by nanoindentation, was found to be different between OP and OA by one study[12] but indifferent by another study.[17]

In contrast to trabecular bone, there were subtle variations in the TMD of subchondral plate in relation to local severity of cartilage degradation. This is in line with Aspden et al.[14] who showed that there was no site-related variation in the material density of subchondral plate, without accounting for condition of the overlying cartilage. In another study[15] they also reported values for trabecular bone but a comparison with subchondral plate was missing. Our study provided new data showing that the TMD of the subchondral plate was significantly lower than that of trabecular bone in OP and OA, and this compartmental difference varied significantly between regions with varying severity of cartilage degradation. The compartmental difference of TMD is consistent with Cox et al.[25] who showed decreased subchondral trabecular bone mineralization toward articular surface. Our results also indicate that such depth-related difference is not OA-specific, but also exists in OP. However, Cox et al. reported that the cartilage degradation-related regional difference of trabecular TMD was larger toward articular surface, whereas the regional difference of subchondral plate TMD in our study was not significant. Taken together, our results support the concept that the subchondral plate and trabecular bone are biologically and mechanically distinct,[2,38] and provide further evidence for the view that they respond differently during the progression of OA.[9,30]

The regional comparisons of microarchitecture in OA knees in relation to the stage of cartilage degeneration have been reported by several previous studies.[26,27] Overall, our data from hip OA are consistent with these studies and provide further information regarding how these stagewise differences are related to the properties of subchondral bone in OP. OP and early cartilage degeneration regions in OA showed similarly reduced trabecular bone volume and deteriorated trabecular structure compared to advanced degeneration regions. The difference in subchondral plate thickness between OP and OA was already significant from OA Grade 2, which seems to agree with that in non-traumatic type of OA the change in subchondral plate precedes the change in trabecular bone and couples more closely with cartilage degeneration.[46,47]

Our regionalized analysis of subchondral bone may suggest that before a comparatively normal region progresses to end-stage OA with degraded cartilage and subchondral sclerosis, there is a period when subchondral bone is osteoporosis-like in terms of both microarchitecture and matrix mineralization, in both subchondral plate and trabecular compartment (Figure 2C). This supports the hypothesis that subchondral bone remodeling in OA is a biphasic procedure with a transition from favoring resorption at early stage to favoring formation at late stage.[9,21] We suggest that this transition may be caused by increased shear/tensile stress concentrated at the junction between sclerotic and porotic regions. The elevated mechanical

stress perceived by osteocytes and osteoblasts in this area may trigger the phenotype change described by previous studies,[48–50] switching osteoblasts from pro-resorption to pro-formation. In the meantime, abnormal production of collagen homotrimers and changes in mineralization-related proteins lead to disrupted mineral deposition and consequently hypo-mineralization.[49,50] This theory parallels the hypothesis that the elevated shear stress, rather than compressive stress, is the cause of cartilage deformation and degradation.[4,9]

The biomechanical properties of bone are characterized by a series of parameters at both microstructural and tissue material levels.[51] Of these parameters, BV/TV and TMD are considered the most important for trabecular bone.[10,15,32] They are the determinants of apparent density which is often clinically measured by Dual Energy X-ray Absorptiometry (DXA) or Quantitative CT (QCT) as areal or volumetric BMD,[39,51] and are assumed to have a mutually adaptive relationship to define the overall stiffness of bone.[29,45] Compared to clinical imaging techniques, micro-CT benefits from higher resolution and is able to provide assessment of volumetric BMD, TMD, and more accurate measurement of bone microarchitecture simultaneously, enabling investigation of the relationships among them. Our study showed that there was an inverse correlation between BV/TV and TMD and between BMD and TMD of trabecular bone in OA. Together they indicate that in OA the decreased matrix mineralization can be over-compensated by the increased bone volume and lead to increased apparent density. This is consistent with previous studies showing that decreased mineralization in OA trabecular bone only compromised but did not completely abolish the increase in bone strength.[1,15] The result is also consistent with the report that a 4%–6% decrease in TMD in sclerotic bone samples was responsible for only a 4%–9% increase in BV/TV, much less than the actual change (69%) in BV/TV.[25] Another interesting observation from our study is that the pattern of the mutual correlations among BMD, BV/TV, and TMD in OA remained the same when analyzed separately by the microscopic grades. This finding was unexpected as bone remodeling in each region was expected to be different, as discussed above and in.[23,50] Also, a study reported a nonsignificant correlation between BV/TV and TMD in end-stage OA subchondral trabecular bone with cysts and no cartilage coverage.[52] One possible explanation for our finding is that the mineralization of bone is affected by a complex mechanism involving not just remodeling rate, but also remodeling balance and mineralization kinetics.[53]

We have also shown that the correlations among BV/TV, TMD, and BMD discussed above also exist in the OP group. These correlations may suggest that loss of trabecular bone in OP is associated with, but not compensated by the increased mineral content, leading to a lower apparent density. This is consistent with the findings that the decreased trabecular bone apparent density in OP was accompanied by stronger and denser trabeculae.[54] The increasing TMD in OP trabecular bone can be a result of active response of osteocytes and osteoblasts to counteract the decreasing stiffness caused by the reducing bone volume,[29,45] or a nonspecific phenomenon as the bone surviving resorption was inner, older, and thus better mineralized trabecular laminae.[29]

A limitation of this study is that measurements were made at a single time point, representing spatial differences rather than temporal changes. However, the progression of OA can be indicated by the regionalized progression of cartilage degeneration.[23–25,28] Further studies of specimens from young and age-matched healthy controls, and subjects with early diseases would be required to provide reliable data on disease progression. Another limitation of this type of this study is the assumption of independence of samples,[15,24,25] when multiple plugs were sampled from the same specimen. We made this assumption because the plug collection was based on the condition of the overlying cartilage, and the histological evaluation confirmed that our macroscopic sampling procedure well represented and differentiated between varying degrees of cartilage degeneration. This assumption is supported by the intra-sample variation of bone parameters in relation to regional severity of cartilage degradation observed in this and other previous studies.[23–26] Moreover, the anatomical distribution of cartilage degeneration and subchondral bone properties across an OA femoral head varied between our specimens, and this is consistent with a previous study showing that there is no anatomical site in the OA specimens for which the bone properties are systematically different.[55] Accordingly, OP plugs were collected from three representative anatomical sites of each femoral head to compare with OA.

It should also be noted that the TMD calculated from a desktop micro-CT is subjected to beam-hardening artifact and only represents the mean degree of mineralization.[25,39,56] In this regard, techniques such as gravimetric measurement, compositional analysis, quantitative backscatter scanning electron microscopy and calibrated monochromatic synchrotron micro-CT imaging can be used in the future to confirm our results. In addition, the current study focused on the microstructural and mineral properties of subchondral bone, further studies involving mechanical testing should be conducted to explore the relationships between these parameters and bone biomechanical features (stiffness, elastic modulus, etc.) in different disease conditions. Finally, the region of interest in this study was confined to the most subchondral areas, whereas the changes in epiphyseal/metaphyseal bone may also contribute to OA pathogenesis and should be investigated in future studies.

In conclusion, the results of our study suggest that the comparison of subchondral bone between OP and OA should take both regional and compartmental differences into account, at both structural and material level. This may be particularly relevant when studying the mechanical interactions between cartilage and subchondral bone in future studies. Longitudinal and cross-sectional studies are needed to better understand the mechanism of the transition of OA subchondral bone from being porous to being sclerotic, which may hold the key to the development of DMOADs targeting bone.

ACKNOWLEDGMENTS

Yunfei Li is a PhD student supported by a scholarship from the Joint China Scholarship Council—University of Bristol Scholarship Scheme. The micro-CT scanning was carried out at the Skeletal. AI Laboratory

of University of Sheffield, with the help from Mrs Caroline Fry. We are grateful for Dr. Erika Kague and the Histology Services Unit led by Mrs. Debbie Martin at University of Bristol for their invaluable technical support and advice. We also want to thank Professor John Tarlton at University of Bristol, Professor Zaitunnatakhin Zamli at International Islamic University Malaysia for their valuable advice for the study design. In addition, this study would not be possible without the help from Dr. Mike Whitehouse, Dr. Damien Tucker, and Dr. Bas Van Ooij who provided surgical specimens from patients undergoing THA at the Avon Orthopedic Center, Southmead Hospital, Bristol, UK.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Design of study: Sharif, Blom, Li. *Acquisition of data*: Li, Liem, Sullivan, Dall'Ara, Ahmed, Blom. *Analysis and interpretation of data*: Li, Sharif, Liem, Dall'Ara. *Drafting of manuscript*: Li, Sharif. *Critical revision of manuscript*: all authors. *Approval of final manuscript*: all authors.

ORCID

Yunfei Li https://orcid.org/0000-0002-5316-2420
Mohammed Sharif https://orcid.org/0000-0001-9723-6295

REFERENCES

1. Dequeker J, Aerssens J, Luyten FP. Osteoarthritis and osteoporosis: clinical and research evidence of inverse relationship. *Aging Clin Exp Res*. 2003;15:426-439.
2. Li G, Yin J, Gao J, et al. Subchondral bone in osteoarthritis: insight into risk factors and microstructural changes. *Arthritis Res Ther*. 2013;15:223.
3. Lajeunesse D, Pelletier J, Martel-Pelletier J. Osteoporosis and osteoarthritis: bone is the common battleground. *Medicographia*. 2010; 32:391-395.
4. Goldring SR, Goldring MB. Changes in the osteochondral unit during osteoarthritis: structure, function and cartilage-bone crosstalk. *Nat Rev Rheumatol*. 2016;12:632-644.
5. Radin EL, Paul IL, Tolkoff MJ. Subchondral bone changes in patients with early degenerative joint disease. *Arthritis Rheum*. 1970;13: 400-405.
6. Calvo E, Castaneda S, Largo R, Fernández-Valle M, Rodriguez-Salvanes F, Herrero-Beaumont G. Osteoporosis increases the severity of cartilage damage in an experimental model of osteoarthritis in rabbits. *Osteoarthritis Cartilage*. 2007;15:69-77.
7. Bellido M, Lugo L, Roman-Blas JA, et al. Subchondral bone microstructural damage by increased remodelling aggravates experimental osteoarthritis preceded by osteoporosis. *Arthritis Res Ther*. 2010;12:R152.
8. Bettica P, Cline G, Hart DJ, Meyer J, Spector TD. Evidence for increased bone resorption in patients with progressive knee osteoarthritis: longitudinal results from the Chingford study. *Arthritis Rheum*. 2002;46:3178-3184.
9. Burr DB, Gallant MA. Bone remodelling in osteoarthritis. *Nat Rev Rheumatol*. 2012;8:665-673.
10. Day J, Ding M, Van Der Linden J, Hvid I, Sumner D, Weinans H. A decreased subchondral trabecular bone tissue elastic modulus is associated with pre-arthritic cartilage damage. *J Orthop Res*. 2001; 19:914-918.
11. Dall'Ara E, Öhman C, Baleani M, Viceconti M. Reduced tissue hardness of trabecular bone is associated with severe osteoarthritis. *J Biomech*. 2011;44:1593-1598.
12. Coats AM, Zioupos P, Aspden RM. Material properties of subchondral bone from patients with osteoporosis or osteoarthritis by microindentation testing and electron probe microanalysis. *Calcif Tissue Int*. 2003;73:66-71.
13. Mansell JP, Bailey AJ. Abnormal cancellous bone collagen metabolism in osteoarthritis. *J Clin Invest*. 1998;101:1596-1603.
14. Li BH, Aspden RM. Mechanical and material properties of the subchondral bone plate from the femoral head of patients with osteoarthritis or osteoporosis. *Ann Rheum Dis*. 1997;56:247-254.
15. Li BH, Aspden RM. Composition and mechanical properties of cancellous bone from the femoral head of patients with osteoporosis or osteoarthritis. *J Bone Miner Res*. 1997;12:641-651.
16. Hart DJ, Mootoosamy I, Doyle DV, Spector TD. The relationship between osteoarthritis and osteoporosis in the general population: the Chingford Study. *Ann Rheum Dis*. 1994;53:158-162.
17. Li ZC, Dai LY, Jiang LS, Qiu S. Difference in subchondral cancellous bone between postmenopausal women with hip osteoarthritis and osteoporotic fracture: implication for fatigue microdamage, bone microarchitecture, and biomechanical properties. *Arthritis Rheum*. 2012;64:3955-3962.
18. Venäläinen MS, Mononen ME, Jurvelin JS, Töyräs J, Virén T, Korhonen RK. Importance of material properties and porosity of bone on mechanical response of articular cartilage in human knee joint—a two-dimensional finite element study. *J Biomech Eng*. 2014; 136(12).
19. Amini M, Nazemi SM, Lanovaz JL, et al. Individual and combined effects of OA-related subchondral bone alterations on proximal tibial surface stiffness: a parametric finite element modeling study. *Med Eng Phys*. 2015;37:783-791.
20. Bultink IE, Lems WF. Osteoarthritis and osteoporosis: what is the overlap? *Curr Rheumatol Rep*. 2013;15:328.
21. Castaneda S, Roman-Blas JA, Largo R, Herrero-Beaumont G. Subchondral bone as a key target for osteoarthritis treatment. *Biochem Pharmacol*. 2012;83:315-323.
22. Burnett WD, Kontulainen SA, McLennan CE, et al. Proximal tibial trabecular bone mineral density is related to pain in patients with osteoarthritis. *Arthritis Res Ther*. 2017;19:1-9.
23. Klose-Jensen R, Hartlev LB, Boel LWT, et al. Subchondral bone turnover, but not bone volume, is increased in early stage osteoarthritic lesions in the human hip joint. *Osteoarthritis Cartilage*. 2015;23:2167-2173.
24. Chappard C, Peyrin F, Bonnassie A, et al. Subchondral bone microarchitectural alterations in osteoarthritis: a synchrotron microcomputed tomography study. *Osteoarthritis Cartilage*. 2006;14: 215-223.
25. Cox LGE, van Donkelaar CC, van Rietbergen B, Emans PJ, Ito K. Decreased bone tissue mineralization can partly explain subchondral sclerosis observed in osteoarthritis. *Bone*. 2012;50: 1152-1161.
26. Finnilä MAJ, Thevenot J, Aho OM, et al. Association between subchondral bone structure and osteoarthritis histopathological grade. *J Orthop Res*. 2017;35:785-792.
27. Bobinac D, Spanjol J, Zoricic S, Maric I. Changes in articular cartilage and subchondral bone histomorphometry in osteoarthritic knee joints in humans. *Bone*. 2003;32:284-290.
28. Muratovic D, Findlay DM, Cicuttini FM, et al. Bone marrow lesions in knee osteoarthritis: regional differences in tibial subchondral bone microstructure and their association with cartilage degeneration. *Osteoarthritis Cartilage*. 2019;27:1653-1662.

29. Van der Linden J, Day J, Verhaar J, Weinans H. Altered tissue properties induce changes in cancellous bone architecture in aging and diseases. *J Biomech*. 2004;37:367-374.
30. Cucchiarini M, de Girolamo L, Filardo G, et al. Basic science of osteoarthritis. *J Exp Orthop*. 2016;3:22.
31. Bobinac D, Marinovic M, Bazdulj E, et al. Microstructural alterations of femoral head articular cartilage and subchondral bone in osteoarthritis and osteoporosis. *Osteoarthritis Cartilage*. 2013;21:1724-1730.
32. Zhang Z-M, Li Z-C, Jiang L-S, Jiang S-D, Dai L-Y. Micro-CT and mechanical evaluation of subchondral trabecular bone structure between postmenopausal women with osteoarthritis and osteoporosis. *Osteoporos Int*. 2010;21:1383-1390.
33. Zhang Z-M, Jiang L-S, Jiang S-D, Dai L-Y. Differential articular calcified cartilage and subchondral bone in postmenopausal women with osteoarthritis and osteoporosis: two-dimensional analysis. *Joint Bone Spine*. 2009;76:674-679.
34. Qaseem A, Forciea MA, McLean RM, Denberg TD. Treatment of low bone density or osteoporosis to prevent fractures in men and women: a clinical practice guideline update from the American College of Physicians. *Ann Intern Med*. 2017;166:818-839.
35. Camacho PM, Petak SM, Binkley N, et al. American Association of Clinical Endocrinologists/American College of Endocrinology clinical practice guidelines for the diagnosis and treatment of postmenopausal osteoporosis—2020 update. *Endocr Pract*. 2020;26:1-46.
36. Pritzker KPH, Gay S, Jimenez SA, et al. Osteoarthritis cartilage histopathology: grading and staging. *Osteoarthritis Cartilage*. 2006;14:13-29.
37. Van den Borne MPJ, Raijmakers NJH, Vanlauwe J, et al. International Cartilage Repair Society (ICRS) and Oswestry macroscopic cartilage evaluation scores validated for use in Autologous Chondrocyte Implantation (ACI) and microfracture. *Osteoarthritis Cartilage*. 2007;15:1397-1402.
38. Madry H, van Dijk CN, Mueller-Gerbl M. The basic science of the subchondral bone. *Knee Surg Sports Traumatol Arthrosc*. 2010;18:419-433.
39. Bouxsein ML, Boyd SK, Christiansen BA, Guldberg RE, Jepsen KJ, Muller R. Guidelines for assessment of bone microstructure in rodents using micro-computed tomography. *J Bone Miner Res*. 2010;25:1468-1486.
40. Mashiatulla M, Ross RD, Sumner DR. Validation of cortical bone mineral density distribution using micro-computed tomography. *Bone*. 2017;99:53-61.
41. Pauli C, Whiteside R, Heras FL, et al. Comparison of cartilage histopathology assessment systems on human knee joints at all stages of osteoarthritis development. *Osteoarthritis Cartilage*. 2012;20:476-485.
42. Roschger P, Rinnerthaler S, Yates J, Rodan G, Fratzl P, Klaushofer K. Alendronate increases degree and uniformity of mineralization in cancellous bone and decreases the porosity in cortical bone of osteoporotic women. *Bone*. 2001;29:185-191.
43. Busse B, Hahn M, Soltau M, et al. Increased calcium content and inhomogeneity of mineralization render bone toughness in osteoporosis: mineralization, morphology and biomechanics of human single trabeculae. *Bone*. 2009;45:1034-1043.
44. Osterhoff G, Morgan EF, Shefelbine SJ, Karim L, McNamara LM, Augat P. Bone mechanical properties and changes with osteoporosis. *Injury*. 2016;47:S11-S20.
45. McNamara L. Perspective on post-menopausal osteoporosis: establishing an interdisciplinary understanding of the sequence of events from the molecular level to whole bone fractures. *J R Soc Interface*. 2010;7:353-372.
46. Goldring SR. Alterations in periarticular bone and cross talk between subchondral bone and articular cartilage in osteoarthritis. *Ther Adv Musculoskelet Dis*. 2012;4:249-258.
47. Buckland-Wright J, Lynch J, Dave B. Early radiographic features in patients with anterior cruciate ligament rupture. *Ann Rheum Dis*. 2000;59:641-646.
48. Massicotte F, Lajeunesse D, Benderdour M, et al. Can altered production of interleukin-1β, interleukin-6, transforming growth factor-β and prostaglandin E2 by isolated human subchondral osteoblasts identify two subgroups of osteoarthritic patients. *Osteoarthritis Cartilage*. 2002;10:491-500.
49. Bailey AJ, Sims TJ, Knott L. Phenotypic expression of osteoblast collagen in osteoarthritic bone: production of type I homotrimer. *Int J Biochem Cell Biol*. 2002;34:176-182.
50. Sanchez C, Deberg MA, Bellahcène A, et al. Phenotypic characterization of osteoblasts from the sclerotic zones of osteoarthritic subchondral bone. *Arthritis Rheum*. 2008;58:442-455.
51. Johnston JD, Kontulainen SA, Masri BA, Wilson DR. Predicting subchondral bone stiffness using a depth-specific CT topographic mapping technique in normal and osteoarthritic proximal tibiae. *Clin Biomech*. 2011;26:1012-1018.
52. Chiba K, Nango N, Kubota S, et al. Relationship between microstructure and degree of mineralization in subchondral bone of osteoarthritis: a synchrotron radiation μCT study. *J Bone Miner Res*. 2012;27:1511-1517.
53. Roschger P, Paschalis E, Fratzl P, Klaushofer K. Bone mineralization density distribution in health and disease. *Bone*. 2008;42:456-466.
54. McNamara L, Prendergast P, Schaffler M. Bone tissue material properties are altered during osteoporosis. *J Musculoskelet Neuronal Interact*. 2005;5:342.
55. Ryan M, Barnett L, Rochester J, Wilkinson J, Dall'Ara E. A new approach to comprehensively evaluate the morphological properties of the human femoral head: example of application to osteoarthritic joint. *Sci Rep*. 2020;10:1-10.
56. Kazakia G, Burghardt A, Cheung S, Majumdar S. Assessment of bone tissue mineralization by conventional x-ray microcomputed tomography: comparison with synchrotron radiation microcomputed tomography and ash measurements. *Med Phys*. 2008;35:3170-3179.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Li Y, Liem Y, Dall'Ara E, et al. et al. Subchondral bone microarchitecture and mineral density in human osteoarthritis and osteoporosis: A regional and compartmental analysis. *J Orthop Res*. 2021;1-13.
https://doi.org/10.1002/jor.25018