Microstructural alterations of femoral head articular cartilage and subchondral bone in osteoarthritis and osteoporosis

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SUMMARY

Objective: Explore whether osteoporosis (OP) in humans influences the morphological status of the articular cartilage and the subchondral bone. Explore the relationship between the macroscopic aspect of the articular surface and the rate of microscopic changes of both the cartilage and the subchondral bone in OP and osteoarthritis (OA).

Methods: Femoral heads after total hip replacement were obtained from patients with OP or hip OA (OP, n = 56; OA, n = 12). Cartilage degeneration was assessed using the Mankin grading system whereas subchondral bone was evaluated using histomorphometry and Micro-computed Tomography (μCT) scanning system. Thickness of the cartilage layers and subchondral cortical bone (SCB) was measured.

Results: Samples with higher total Mankin score have significantly reduced cartilage thickness. Mankin score differed between all OP specimens. In OP samples with lower Mankin scores the thickness of SCB shows a trend of an increase caused by increased levels of bone remodeling. In OP samples with higher Mankin scores we observed thinning of SCB. Structural indices of subchondral trabecular bone (STB) were significantly lower in OP than in OA samples.

Conclusion: Thinning of SCB, found in OP samples with higher Mankin scores could be related with the progression of the cartilage degeneration indicating an early-stage OA. Increased levels of bone remodeling and evidently changed morphology of subchondral bone found in OP samples with lower Mankin score indicated that bony bed level must have a role in the progression of the cartilage degeneration.

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Introduction

Osteoarthritis (OA) and osteoporosis (OP) are two common age-related skeletal disorders. OA is considered a disease of the articular cartilage and the subchondral bone. Thus, severe cartilage damage, sclerotic subchondral bone and osteophyte formation are the main features in osteoarthritic joints. In turn, OP is considered to be a disease of bone characterized by a compromised bone strength and increased bone compliance due to the loss of trabecular bone. Patients with postmenopausal OP and those with OA represent anthropometrically different populations what support clinical aspects of inverse relationship between OP and OA. Epidemiological surveys suggest that OA and OP are rarely present together in the same patient. Other studies suggest that the presence of one disease may be considered protective against the other. That is, an increase in bone compliance may offer a protective effect for the articular cartilage and therefore the articular cartilage in OP remains unchanged. However, two recent studies on animal models, showed a detrimental effect of OP on articular cartilage, bringing the protective effect of OP on OA into question. These recent studies in animals highlight the importance of further research and understanding of the articular cartilage and the underlying bone layers in OP in humans.

There are many reports about severe microscopic changes of the cartilage and subchondral bone in the late-stage OA in humans and in animal models. About early-stage OA there are few reports exclusively in animal models. On the other hand, about OP there are many reports about microscopic changes of the trabecular
bone in humans and in animal models, but very few about over-lying articular cartilage5,15,16.

In late-stage OA there was an increase in subchondral bone volume and decrease in material density signifying lower bone mineralization and the mechanical stiffness9. Therefore, sub-chondral plate was thicker10,11,17,18. In OA human studies, an in-crease in trabecular bone volume fraction (BV/TV) and trabecular thickness (Tb.Th) were reported15,18,19. Contrary, some reports revealed a lower BV/TV and decrease of Tb.Th in patients with OA20,21. However, Chappard et al. reported that areas with high and low BV/TV depend on the condition of the overlying cartilage22.

Little attention has been paid to the articular cartilage and SCB in patients with OP. This is our focus here. We hypothesize that OP influences the cartilage and SCB and can be associated with severe cartilage damage. Also we were interested in relation between macroscopic appearance and microscopic changes of the articular cartilage in patients affected either by OP or OA.

Material and methods

Donor selection

In compliance with institutional safety and ethics regulations, human femoral heads were obtained after surgical removal from patients undergoing a hip replacement for a fractured neck of femur attributed to OP or for OA of the hip. The samples were ob-tained from 56 patients (37 female and 19 male, mean age 78 years, range 50–98) with femoral neck fracture and from 12 patients (seven female and five male, mean age 68 years, range 40–78) with primary hip OA. The research was approved by the Ethics Com-mittee of the School of Medicine University of Rijeka and Clinical Hospital Rijeka. All patients signed an informed consent.

OP fracture was defined as a fracture without preceding trauma or in response to a minimal trauma. Patients suffering from hip fractures following severe traumas, patients with known diseases that could interfere with bone metabolism (such as osteomalacia, multiple myeloma, rheumatoid arthritis, secondary OP due to corticosteroids), and patients treated with bisphosphonates in the last year before inclusion were excluded from the osteoporotic group. Patients with rheumatoid arthritis and osteomalacia were excluded from the osteoarthritic group.

Macroscopic examination

The surface of the femoral articular cartilage at the weight bearing area was macroscopically inspected by two blind observers. The assessment was performed using a semi quantitative scale23. The severity of macroscopic changes was categorized as follows:

Grade 0 – normal cartilage with smooth and glossy surface pale colored;
Grade I – discoloration, mild surface irregularities, cartilage softening and swelling;
Grade II – partial-thickness defect and fibrillation;
Grade III – full-thickness erosion and fissuring to the level of the subchondral bone.

After macroscopic examination, a cylindrical core (20 mm in length and 8 mm in diameter) of the articular cartilage and sub-chondral bone was extracted from each femoral head at the weight bearing area, i.e., the superior part of the femoral head for measurement. The cartilage-subchondral bone specimens were drilled out with a stainless steel trephine. The axes of the specimens were perpendicular to the articular surface. The specimens were stored in ethanol (70%) prior to testing.

Histology

For histological examination, each core specimen was fixed in buffered formalin solution for 24 h and decalcified for 6 weeks in 10% ethylene-diamino-tetracetic acid (EDTA) solution. Next, specimens were embedded in paraffin wax. Longitudinal, 3 μm thick serial sections of cartilage-bone cylinders were cut by a microtome (RM550, Leica rotary microtome, Vienna, Austria). Sections were stained using two methods: the Safranin-O (SO) method, which enables the assessment of structural changes of the articular cartilage and the proteoglycan content in extracellular matrix; and Goldner-trichrome method to evaluate the sub-chondral bony structures and osteoid.

The sections of the articular cartilage stained with SO method were histopathologically assessed by an experienced pathologist using Mankin’s grading system23. The observer was blinded with respect to the diagnosis and macroscopic changes of the articular surface. A partial score for each Mankin’s scale category (i.e., cartilage structure abnormalities, cellularity, alterations in the level of SO staining and tidemark integrity) was allocated and the scores in each of these categories were counted for every section.

Three mineralized tissues can be differentiated in the joint: calcified cartilage (CC), subchondral cortical bone (SCB) and sub-chondral trabecular bone (STB)24. Superficial layers of the articular cartilage are non-calcified while the deepest layer is calcified. The boundary between calcified and non-calcified cartilage (NCC) is referred to as the tidemark. CC is a thin, avascular and highly mineralized (but not ossified) layer8. Next deeper layer is SCB. The interface between CC and SCB is transitional line. SCB is corticalized, not very porous and may not be very vascular, although provides the vascular supply for the deepest cartilage layers. SCB may change its density and thickness through bone remodeling or direct apposition of bone to its distal surface through process called modeling25. Supporting SCB from beneath is the layer of STB. STB is anisotropic and contains haemopoetic bone marrow with blood vessels.

Measurement of the thickness of NCC, CC and SCB

All measurements were carried out at the sections stained by Goldner-trichrome method under 400× magnification and the results were expressed in micrometers. The thickness of the articular cartilage was defined as the mean perpendicular distance from the articular surface to the tidemark. The thickness of CC was defined as the mean perpendicular distance from the tidemark to the transitional line of CC and SCB. The thickness of SCB was defined as the mean perpendicular distance from the transitional line of CC and SCB to manually delineated transitional line of the STB and the bone marrow. Since the thickness of all three layers was measured at several points, the thickness of each layer was calculated as the mean value of all the measurements.

Histomorphometry

Bone histomorphometry of SCB layer was performed using image analysis software (software SFOR, VAMS, Zagreb, Croatia). All images were obtained using a digital camera of a light microscope (Olympus BX50, Tokyo, Japan) at 400× magnification (Olympus, Tokyo, Japan). Following parameters were measured: BV/TV, in % and porosity which describes the ratio of the volume of the pores over the total volume. Bone histomorphometry can provide 3D data on bone structure, bone quality and levels of bone turnover, which cannot be obtained from other methods.

Micro-computed Tomography (μCT) was used to calculate 3D structural parameters of STB. All samples were scanned using a
high-resolution Skyscan μCT scanner (Skyscan 1076, Aartselaar, Belgium). The X-ray source was set at 50 kV with a pixel size of 18 μm. Three hundred and thirty projections were acquired over an angular size 360° (angular step of 0.60°). Image slices were reconstructed using the NRecon software (Skyscan, Aartselaar, Belgium). The trabecular bone was extracted by drawing round contours with CTAn software (Skyscan, Aartselaar, Belgium) and selected regions of interest were analyzed using the same application. Following parameters were measured: BV/TV, Tb.Th, trabecular number (Tb.N) and trabecular separation (Tb.Sp).

Statistical analysis

Results are expressed as mean and standard deviations of the mean. Data from multiple groups were compared using Kruskal–Wallis and Mann–Whitney non-parametric analyses, as appropriate. Differences were considered significant when \( P < 0.05 \).

Results

Gross pathology

We obtained 68 femoral heads from patients who underwent the hip arthroplasty, either because of the femoral neck fracture (56) or hip OA (12). One of OP samples was obtained from a donor who previously suffered from hip OA. The results of the macroscopic assessment of the articular surface are shown in Table I, separately for OP and OA samples. The distribution of the results of the macroscopic assessment and the total Mankin scores for each cartilage is shown in Table II. The range of overall Mankin scores varied between 4 and 13 for every specimen. The results concerning OA specimens showed unique pattern such as the highest total Mankin scores and the grade of macroscopic assessment, respectively (Table II). However, the results related to all OP specimens were very different. Only in 24 of 56 OP femoral heads the articular surface at the weight bearing area appeared normal (Grade 0) (Tables I and II). However, later histological analysis of the cartilage revealed degenerative changes, e.g., diffuse hipercellularity and moderate reduction in SO staining (Mankin scores 4–9). Markedly, in 14 of 24 samples the tidemark was already breached by blood vessels [Fig. 1(A)].

Next 20 of 56 OP specimens with softening and swelling at the cartilage surface were assessed as Grade I. The total Mankin scores in this group were 6–9 (Table II). In almost every specimen the tidemark was interrupted by blood vessels [Fig. 1(B)].

Seven of 56 OP samples were furthermore assessed as Grade II, because of the fibrillation at the articular surface. In these cases histological analysis showed stronger structural and degenerative changes of the cartilage with the overall Mankin scores between 10 and 12. Structural irregularities consisted of clefts that reached the transitional and radial zones. Chondrocytes were grouped in clones and matrix staining intensity was reduced. The tidemark was irregular and also breached by blood vessels.

Finally five of 56 OP specimens were assessed following macroscopic evaluation as Grade III because there was complete loss of the cartilage (Table I and II). In both OP and OA samples of Grade III histological analysis showed a loss of cartilage thickness, fissures next to the deep calcified zone, hypercellularity, cells grouped in clones, and severe reduction of extracellular matrix staining. The tidemark was duplicated and was breached by blood vessels [Fig. 1(C)].

With respect to the overall Mankin scores the samples with Mankin score 0–9 were predominately OP samples while the samples with Mankin score 10–13 were both OP and OA samples, represented in equal proportion (Table III). The samples with lower score had significantly thicker (\( P < 0.001 \)) NCC layer than the samples with higher Mankin scores. There was no significant difference in SCB thickness between both groups according to Mankin scores but SCB in the samples with lower Mankin scores have tendency to be thicker [Fig. 1(D)]. In these samples SCB was characterized by the development of new Haversian channels and hipercellularity of newly formed bone, caused by altered bone remodeling with increased rate of bone formation and resorption [Fig. 1(A and B)]. Related to the bone histomorphometric parameters the samples with lower total Mankin scores had a significantly lower BV/TV (\( P < 0.001 \)) than samples with higher Mankin scores (Table III). Significant differences were not found in Tb.Th, Tb.N and Tb.Sp between these two groups of samples.

### Table I

<table>
<thead>
<tr>
<th>Grade</th>
<th>0 (n = 24)</th>
<th>1 (n = 21)</th>
<th>II (n = 7)</th>
<th>III (n = 16)</th>
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<td>Grade</td>
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<td>2 OA</td>
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<td>(tm-I)</td>
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<td>(tm-I)</td>
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</tbody>
</table>

Note: tm – tidemark, N – normal appearance of tidemark, I – irregular tidemark breached by blood vessels.

### Table II

The distribution of the results of macroscopic assessment of the articular surface and the corresponding total Mankin score of each specimen

<table>
<thead>
<tr>
<th>Grade</th>
<th>0 (n = 24)</th>
<th>1 (n = 21)</th>
<th>II (n = 7)</th>
<th>III (n = 16)</th>
</tr>
</thead>
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<td>II (n = 7)</td>
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</tr>
<tr>
<td></td>
<td>1 OP</td>
<td>1 OP</td>
<td>1 OA</td>
<td>2 OA</td>
</tr>
<tr>
<td></td>
<td>(tm-I)</td>
<td>(tm-I)</td>
<td>(tm-I)</td>
<td>(tm-I)</td>
</tr>
</tbody>
</table>
Severe abnormalities of cartilage structure, cellular density, matrix staining and the tidemark integrity were presented in both OP and OA samples with total Mankin scores over 10. In both OP and OA samples, the thickness of the NCC layer was highly reduced (Table IV). Conversely, comparative study between OP and OA samples with total Mankin score over 10 revealed considerable differences in bone parameters (Table IV). The thickness of SCB in OP samples with severe cartilage changes has tendency to be thinner than in OA samples \( (P = 0.127) \) (Table IV). In OA samples SCB was characterized by increase in bone resorption and formation sites [Fig. 2(A and B)]. Furthermore, extended vascular channels with circular layers of newly formed bone in different sizes increased SCB porosity which was significantly higher \( (P < 0.05) \) in OA samples (Table IV) [Fig. 2(B)]. We found greater amount of osteoid in OP than in OA samples [Fig. 1(E and F)].

**Table III**
The comparison between samples with total Mankin score between 0 and 9 and samples with total Mankin score between 10 and 13 for all examined parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mankin 0–9 ( N = 44 ) OP ( + 1 ) OA</th>
<th>Mankin 10–13 ( N = 12 ) OP ( + 11 ) OA</th>
<th>( P )-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>( 78 \pm 11 )</td>
<td>50–98</td>
<td>( 68 \pm 13 )</td>
</tr>
<tr>
<td>Articular cartilage</td>
<td>( 2.24 \pm 0.73 )</td>
<td>0.90–4.38</td>
<td>( 1.22 \pm 0.56 )</td>
</tr>
<tr>
<td>CC thickness (mm)</td>
<td>( 0.11 \pm 0.06 )</td>
<td>0.05–0.21</td>
<td>( 0.13 \pm 0.08 )</td>
</tr>
<tr>
<td>SCB thickness (mm)</td>
<td>( 0.26 \pm 0.18 )</td>
<td>0.05–0.76</td>
<td>( 0.20 \pm 0.14 )</td>
</tr>
<tr>
<td>SCB porosity (%)</td>
<td>( 90.85 \pm 5.63 )</td>
<td>76.1–99.56</td>
<td>( 90.95 \pm 7.06 )</td>
</tr>
<tr>
<td>BV/TV (%)</td>
<td>( 21.91 \pm 5.88 )</td>
<td>12.16–38.08</td>
<td>( 25.61 \pm 11.69 )</td>
</tr>
<tr>
<td>Tb.Th (mm)</td>
<td>( 0.17 \pm 0.02 )</td>
<td>0.13–0.22</td>
<td>( 0.19 \pm 0.04 )</td>
</tr>
<tr>
<td>Tb.N (1/mm)</td>
<td>( 1.25 \pm 0.24 )</td>
<td>0.68–1.74</td>
<td>( 1.32 \pm 0.41 )</td>
</tr>
<tr>
<td>Tb.Sp (mm)</td>
<td>( 0.59 \pm 0.07 )</td>
<td>0.46–0.76</td>
<td>( 0.57 \pm 0.13 )</td>
</tr>
</tbody>
</table>

Note: statistical significant difference at the level \( P < 0.05 \), \( P < 0.001 \) **.
In both OP and OA samples with total Mankin score over 10 all bone parameters of STB were significantly different (Table IV). BV/TV, Tb.Th and Tb.N were significantly lower whereas Tb.Sp was significantly higher in OP than in OA sample group \((P < 0.001)\), respectively (Table IV). These results revealed significant increase of STB mass in OA samples.

**Discussion**

The articular cartilage of all OP samples showed wide range of degenerative changes from the lowest ones which could be considered as aged determined changes\(^{24}\) to the highest ones that are rather similar to the OA cartilage changes. Cartilage thickness was significantly higher in OP samples with lower Mankin scores than in samples with higher Mankin scores. In OP samples with lower degenerative alterations in the cartilage we observed very intensive processes of bone remodeling what presumably resulted in thickening of SCB. Later, with aggravating of the cartilage damage we observed thinning of the SCB. In OA samples we found significantly higher trabecular bone mass than in OP samples although cartilage from both showed severe alterations.

Macroscopic examinations of the articular surface of femoral heads from OP patients revealed, in most cases, either a normal macroscopic appearance or alterations like softening and swelling. Swelling of the cartilage could be early sign of the cartilage alterations because it was initially resulted from proteoglycan degradation activities of the chondrocytes and altered hydration of the cartilage\(^{27}\). Besides, the initial phase of the cartilage hypertrophy, shown in early experimental OA was related to the swelling of the cartilage\(^{14,28–30}\). In even 12/56 femoral heads we found evident alterations of the articular surface which were categorized as Grade II or III. All femoral heads from the patients with late-stage OA of the hip were categorized as Grade III.

Histological analysis of the cartilage in all OP samples have shown alterations in each scale category according Mankin grading system and the range of these changes was from minimal to very severe degree of degeneration, even similar to OA cartilage degeneration. Our results have shown that in 12/56 OP samples the overall Mankin scores were over 10, the same as in OA samples. Some of these OP samples had a signs of fibrillation at the cartilage surface but histological analysis showed severe cartilage degenerative changes. This can be indicative for early-stage OA as has been observed by Ding *et al.* who defined the degree of OA according to the criteria of Mankin and reported that early OA was characterized by the presence of macroscopically fibrillated cartilage\(^{31}\).

Experimentally induced OP, OA and combined OPOA indicated that OP increases the severity of cartilage damage\(^{6,7}\). The overall Mankin scores they obtained from OPOA knees were higher when compared with OA knees. The overall Mankin scores in OA knees were higher than that in OP knees but the differences were not significant unlike OP knees that showed significantly higher scores than normal healthy knees indicating that OP have an aggravating effect on the development of OA lesions.

NCC was significantly thicker in the specimens with lower than with higher overall Mankin scores. Our findings are partly consistent with Zhang *et al.* who have reported that OP patients had insignificantly thicker articular NCC than OA patients\(^{15}\). Franklin *et al.* have exhibited the assessment of hip joint space on radiographs of patients with hip fracture\(^{32}\). They determined in 17% of patients minimal joint space of 2.5 mm or less and even osteophytes which are normally radiographic signs of OA. According to our results 21% of patients with hip fracture had significant loss of cartilage thickness what could be addressed to osteoarthritic lesions.

The OP samples with lower Mankin score have the tendency of lower thickness of CC layer than the samples with higher Mankin score. Increased rate of bone remodeling at the osteochondral junction may decrease CC thickness\(^{33,34}\). These data support our results because we found higher rate of bone remodeling in SCB of samples with lower Mankin score. Zhang *et al.* have reported that OA patients had significantly thicker CC layer compared with the OP patients\(^{15}\). In all our samples with higher total Mankin score and evidently thicker CC layer the tidemark was duplicated. The tidemark duplication or advancement is caused by re-establishment of

**Table IV**
The comparison between OP and OA samples both with total Mankin score from 10 to 13. The comparison was made for each examined parameter of the cartilage, SCB and STB.

<table>
<thead>
<tr>
<th></th>
<th>OP (N = 12/56)</th>
<th>OA (N = 11/12)</th>
<th>P-values</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Range</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Age</td>
<td>75 ± 10</td>
<td>64–91</td>
<td>62 ± 12</td>
</tr>
<tr>
<td>Articular cartilage thickness (mm)</td>
<td>0.13 ± 0.07</td>
<td>0.06–0.23</td>
<td>0.14 ± 0.08</td>
</tr>
<tr>
<td>CC thickness (mm)</td>
<td>1.02 ± 0.42</td>
<td>0.05–2.36</td>
<td>1.07–1.84</td>
</tr>
<tr>
<td>SCB thickness (mm)</td>
<td>2.36 ± 1.02</td>
<td>0.13–3.56</td>
<td>0.20–1.13</td>
</tr>
<tr>
<td>SCB porosity (%)</td>
<td>87.8–98.8</td>
<td>87.7–8.2</td>
<td>70.67–98.79</td>
</tr>
<tr>
<td>BV/TV (%)</td>
<td>108.2–12.32</td>
<td>104.3–9.55</td>
<td>113.4–10.28</td>
</tr>
<tr>
<td>Tb.Th (mm)</td>
<td>0.16 ± 0.03</td>
<td>0.11–0.20</td>
<td>0.05–0.04</td>
</tr>
<tr>
<td>Tb.N (1/mm)</td>
<td>1.09 ± 0.37</td>
<td>0.64–2.01</td>
<td>1.54 ± 0.27</td>
</tr>
<tr>
<td>Tb.Sp (mm)</td>
<td>0.64 ± 0.13</td>
<td>0.43–0.89</td>
<td>0.49 ± 0.08</td>
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</table>

**Fig. 2.** Goldner-trichrome stained longitudinal sections of cartilage-bone samples of femoral heads showing the CC and SCB in OA. (A) thick CC layer with hypertrophic chondrocytes, tidemark is duplicated (two arrows black); resorption pit (asterisk) (B) CC is thin, SCB is thicker with intensive resorption sites (extended vascular channels) (arrow head), vascular channel that penetrates into the NCC (arrow white). (magnification 100×).
the process of endochondral ossification and presumably is in function of the increase of CC thickness\textsuperscript{17,33}. The tidemark breached by blood vessels is usually sign of advanced degenerative changes of the cartilage such as has been reported for OA bone\textsuperscript{34}. However, we found the tidemark breached by blood vessels in OP samples with the overall Mankin score 6–9 and we assume that it is the result of an increased rate of bone turnover in SCB layer with formation of new Haversian channels and blood vessels what can bring about penetration of blood vessels into CC layer.

In SCB of OP samples with lower overall Mankin scores we found newly formed hypercellular bone tissue with new Haversian channels and layers of woven bone. Since new bone formations deeply and irregularly penetrated into CC layer, there was somewhere difficulty to determine the transitional line between these two layers. According to the increased bone turnover, higher thickness of SCB was revealed more in OP samples with lower than in OP samples with higher Mankin score. Because of accelerated bone formation increased amount of osteoid and hypomineralization of adjacent bone tissue was found. The high level of osteoid in OP compared to OA indicated higher metabolic activity and bone turnover in OP samples\textsuperscript{35}. In OA samples SCB was more sclerotic and hypocellular with increased bone resorption activities. Higher thickness of SCB was revealed more in OA than in OP samples what is in accordance with Li and Aspden\textsuperscript{9}. Big pits as bone cysts in SCB resulted in significantly higher porosity of OA samples than OP samples with higher overall Mankin scores. Snickers \textit{et al.} reported that porosity of the subchondral plate increased severely in OA animals\textsuperscript{35}. In altered bone remodeling in late-stage OA as osteoblast regulation of mineralization was impaired, SCB might not be properly mineralized\textsuperscript{17}.

In OP samples with higher overall Mankin scores SCB was mostly thinner, more dense and less porous. Thinning of the subchondral plate was shown in early-stage OA\textsuperscript{12,13}. Considerable reduction in the thickness of the subchondral plate was associated with increased articular cartilage destruction\textsuperscript{5}. In OA animals with previously induced OP (combined OPOA group) there were significant decrease in subchondral plate thickness and considerably more severe cartilage alteration when compared with OP or OA groups. Thus, the thinning of the SCB may be as a sign of developing OA and may be responsible for progression of the cartilage degenerative changes\textsuperscript{17}. In humans with early-stage OA, but without clinical symptoms, resorption markers were elevated\textsuperscript{16}. The increased rate of bone remodeling in early-stage OA may cause alteration in load transmission that predisposes to progressive cartilage loss\textsuperscript{36,37}.

Our study revealed significant difference in STB parameters between samples with lower and higher overall Mankin scores and between OP and OA samples, both with higher Mankin score suggesting poor bone quality in OP samples and higher bone mass in OA samples. OP samples had significantly lower BV/TV, Tb.Th, Tb.N and higher Tb.Sp in comparison to OA samples. Studies on human bone samples revealed significant differences and inverse relationship in microstructure of STB between OP and OA samples\textsuperscript{15,35}.

We have still some limitations in our study. First, the OA sample size for this study was small. However, we had strict criteria for patient inclusion and all patients had end stage of OA with previously evident clinical and radiographic signs. All analysis showed that OA sample group was homogenous according to stage of degenerative changes in the cartilage and subchondral bone. Secondly, we have no completely normal control population because it is difficult to obtain age- and sex-matched bone samples without any diseases.

In summary, our study shows considerable deteriorations of the articular cartilage and SCB in OP in humans. We observed that patients with OP have lower bone mass at the level of STB but intensive changes found in SCB and articular cartilage could signify early signs of developing hip OA in the same patient.

**Author's contributions**

- Dragica Bobinac: conception and design of the study, drafting of the article, final approval of the article, critical revision of the article for important intellectual content.
- Marin Marinovic: collection and assembly of data, provision of study materials and patients.
- Edo Bazdulj: analysis and interpretation of the data.
- Olga Cvijanovic: statistical expertise, drafting of the article.
- Tanja Celic: analysis and interpretation of the data, drafting of the article.
- Ivana Maric: drafting of the article.
- Josip Spanjol: drafting of the article.
- Tedi Cicvaric: conception and design.

All authors approved the final version of the manuscript to be published.

**Conflict of interest statement**

None declared.

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