Original Full Length Article

Decreased bone tissue mineralization can partly explain subchondral sclerosis observed in osteoarthritis

L.G.E. Cox, C.C. van Donkelaar, B. van Rietbergen, P.J. Emans, K. Ito

Abstract

For many years, pharmaceutical therapies for osteoarthritis (OA) were focused on cartilage. However, it has been theorized that bone changes such as increased bone volume fraction and decreased bone matrix mineralization may play an important role in the initiation and pathogenesis of OA as well. The mechanisms behind the bone changes are subject of debate, and a better understanding may help in the development of bone-targeting OA therapies. In the literature, the increase in bone volume fraction has been hypothesized to result from mechanoregulated bone adaptation in response to decreased mineralization. Furthermore, both changes in bone volume fraction and mineralization have been reported to be highest close to the cartilage, and bone volume fraction has been reported to be correlated with cartilage degeneration. These data indicate that cartilage degeneration, bone volume fraction, and bone matrix mineralization may be related in OA. In the current study, we aimed to investigate the relationships between cartilage degeneration, bone matrix mineralization and bone volume fraction at a local level. With microCT, we determined bone matrix mineralization and bone volume fraction as a function of distance from the cartilage in osteochondral plugs from human OA tibia plateaus with varying degrees of cartilage degeneration. In addition, we evaluated whether mechanoregulated bone adaptation in response to decreased bone matrix mineralization may be responsible for the increase in bone volume fraction observed in OA. For this purpose, we used the experimentally obtained mineralization data as input for bone adaptation simulations. We simulated the effect of mechanoregulated bone adaptation in response to different degrees of mineralization, and compared the simulation results to the experimental data. We found that local changes in subchondral bone mineralization and bone volume fraction only occurred underneath severely degenerated cartilage, indicating that bone mineralization and volume fraction are related to cartilage degeneration at a local level. In addition, both the experimental data and the simulations indicated that a depth-dependent increase in bone volume fraction could be caused by decreased bone matrix mineralization. However, a quantitative comparison showed that decreased mineralization can only explain part of the subchondral sclerosis observed in OA.

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Introduction

Osteoarthritis (OA) is a complex multi-factorial joint disease, characterized by degeneration of cartilage and modification of the structural and material properties of subchondral bone [1]. For many years, pharmaceutical therapies have been focused on cartilage, because the bone changes were thought to occur secondary to cartilage degeneration, and not to play a major role in the disease process. However, currently the bone is being reconsidered as a therapeutic target [2,3]. The bone can be a source of pain in OA, and in addition it has been theorized that bone changes may play an important role in the initiation and pathogenesis of OA [2-5].

The most frequently discussed bone change observed in OA is probably subchondral sclerosis. Subchondral sclerosis has been described in many clinical [6-10] and animal studies [11-14]. Radin et al. [15] were the first to suggest that bone sclerosis may play an important role in cartilage degeneration. They hypothesized that sclerosis would lead to a stiffer subchondral structure with a decreased shock-absorbing capability, thereby increasing the stress in the overlying cartilage [15]. Alternatively, the sclerotic bone may influence cartilage degeneration via the release of biochemical signals. Due to the increased bone metabolism associated with the sclerosis [4,16-21], various cytokines, including insulin-like growth factors, transforming growth factor β, and bone morphogenic proteins, which are known to modulate chondrocyte proliferation and differentiation, and matrix synthesis, are released [5,22,23]. In addition to bone volume fraction, bone material properties are altered in OA. Bone matrix mineralization is lower [6,18,24,25] and as a result the material stiffness of OA bone is decreased [18,24,26]. In contrast with the theory of Radin et al., it has been suggested that this
decrease in bone material stiffness may increase cartilage deformation and induce cartilage degeneration upon joint loading [19,27].

Sclerosis and bone matrix mineralization may be closely related, as the increase in bone volume fraction has been hypothesized to result from bone adaptation in response to decreased mineralization [26,28]. In a previous simulation study we showed that bone adaptation may indeed increase bone volume fraction to counteract an increase in bone tissue strain resulting from decreased bone material stiffness [29]. There are also indications that bone volume fraction and mineralization are related to cartilage degeneration. Both bone matrix mineralization [25,30], and bone structure [7] have been reported to change most markedly directly underneath the cartilage, and bone structural changes seemed correlated with the degree of degeneration of the overlying cartilage [7]. A possible explanation for these findings could be that biochemical signaling from degenerating cartilage may influence bone mineralization and/or bone volume fraction.

In the current study, we aimed to investigate the relationships between cartilage degeneration, bone matrix mineralization, and bone volume fraction at a local level. For this purpose, we determined bone matrix mineralization (tissue mineralization, TMD) and bone volume fraction (BV/TV) using microCT, at different depth levels in human osteochondral plugs with varying degrees of cartilage degeneration. These plugs were harvested from OA tibia plateaus obtained after total knee replacement. In addition, we used the experimentally obtained mineralization data as input for bone adaptation simulations, to predict the effect of bone mineralization on bone volume fraction. With this combined experimental–mathematical approach, we aimed to evaluate whether the differences in bone volume fraction observed for different OA grades could be explained by bone adaptation in response to changes in matrix mineralization.

**Methods**

**Sample preparation**

We examined 32 human OA tibia plateaus, obtained after total knee replacement (University hospital Maastricht, Maastricht, The Netherlands). With a diamond coated bit, we cored cylindrical specimens perpendicular to the articular surface, with a diameter of 8 mm and variable depth, dependent on the height of the tibia plateaus. Preferably, one specimen was analyzed for each side (medial and lateral) of the plateau (Fig. 1a). However, for 11 plateaus, specimens from one side could not be used for further analysis, due to either limited height of the plateaus as a result of the location of the surgical cutting plane or the presence of cysts, leading to a total number of 53 bone-cartilage specimens (25 medial, 28 lateral).

**MicroCT**

Each specimen was fixed in formalin and scanned with a microCT scanner (vivaCT40, Scanco Medical AG) at an isotropic voxel size of 21 μm (70 kVp, 114 μA, 500 projections per 180 degrees, 300 ms integration time). A beam-hardening correction algorithm was applied to all scans. The computed linear attenuation coefficient of the X-ray beam in each voxel, which can be considered to be proportional to the local degree of mineralization, was stored in an attenuation map and represented by a gray value in the reconstruction.

Image processing included Gaussian filtering (sigma = 0.8, support = 1) and segmentation with a constant threshold value to discriminate between bone and soft tissue. The segmentation was visually checked. The degree of mineralization for a specific volume of interest of the segmented images was quantified by comparing the attenuation coefficients of the tissue quantified as bone with reference measurements of a phantom containing hydroxyapatite of different densities. For these measurements, the outermost voxel layer characterized as bone was removed, as it is likely to be corrupted by partial volume effects.

Bone volume fraction (BV/TV) and bone matrix mineralization (TMD) were determined for 2 mm thick bone slices parallel to the cartilage using the Scanco Medical AG software. Analyzing 2 mm thick slices is necessary for accurate measurement of structural parameters. To detect depth-dependent changes in bone volume fraction and bone matrix mineralization, we evaluated slices at 5 different distances from the articular cartilage, which will be referred to according to their mean distance as 1, 1.5, 2, 2.5, and 3 mm depth. This means that the bone volume fraction and bone matrix mineralization that we determined at 1 mm depth, are the average values determined for the bone slice between 0 mm and 2 mm distance from the cartilage (Fig. 1b). It also implies that there was a 1.5 mm overlap between consecutive slices. Due to the limited height of some of the tibia plateaus, we could not obtain measurements at all five depth levels for all 53 specimens, leading to a decreasing number of measurements with increasing depth level.

**Cartilage degeneration**

All specimens were dehydrated in graded ethanol solutions (70% to 100%) and embedded in polymethylmethacrylate (PMMA). The embedded specimens were bisected perpendicular to the cartilage using a bandsaw, and then sectioned using a microtome (Leica RM2165, Leica Microsystems), thereby exposing the trabecular microstructure at the surface of the specimens. These surfaces were stained with toluidine blue for 1.5 h and then rinsed in running tap water. Images were

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**Fig. 1.** Sample preparation. (a) Schematic representation of a tibia plateau indicating possible sample locations. (b) Schematic representation of an osteochondral plug indicating the 2 mm slice used to determine mineralization and bone volume fraction values at 1 mm depth.
The Kellgren–Lawrence (K–L) score was used to classify the samples according to their OA grade. The ICRS cartilage degeneration scoring system, as described by Marticke et al. [31] (Table 1).

<table>
<thead>
<tr>
<th>ICRS grade</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>Normal intact cartilage</td>
</tr>
<tr>
<td>1</td>
<td>Chondral softening and blistering, superficial lesions, fissures and cracks, soft indentation</td>
</tr>
<tr>
<td>2</td>
<td>Fraying, lesions and fissures extending down to &lt;50% of cartilage depth</td>
</tr>
<tr>
<td>3</td>
<td>Partial loss of cartilage thickness, cartilage defects extending down to &gt;50% of cartilage depth as well as down to calcified layer</td>
</tr>
<tr>
<td>4</td>
<td>Full-thickness cartilage loss with exposure of the subchondral bone</td>
</tr>
</tbody>
</table>

Table 2 – K-L osteoarthritis scoring system.

<table>
<thead>
<tr>
<th>K–L grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal bone, no joint space narrowing</td>
</tr>
<tr>
<td>1</td>
<td>Doubtful narrowing of joint space and possible osteophytic lipping</td>
</tr>
<tr>
<td>2</td>
<td>Definite narrowing of joint space, osteophytes</td>
</tr>
<tr>
<td>3</td>
<td>Definite narrowing of joint space, osteophytes, some sclerosis and possible deformity of bone contour</td>
</tr>
<tr>
<td>4</td>
<td>Marked narrowing of joint space, large osteophytes, severe sclerosis and definite deformity of bone contour</td>
</tr>
</tbody>
</table>

Table 1 – ICRS cartilage degeneration scoring system.

Fig. 2. Mathematical model. (a) Schematic representation of the bone remodeling theory, adapted from Huiskes et al. [32]. (b) A two-dimensional finite element mesh representing articular cartilage and subchondral bone tissue.
The bone was allowed to remodel for 5000 increments, after which a stable trabecular structure was obtained, that did not change significantly with respect to bone volume fraction, trabecular number, trabecular thickness, and trabecular separation if remodeling was continued for 500 additional increments.

To evaluate whether the differences in bone volume fraction observed for different OA grades can be explained by the differences in matrix mineralization, we used the experimentally determined mineralization data as input for the model. In 1988, Currey found that the relation between bone mineralization and elastic modulus can be approximated with a cubic power law [40]. This power law is included in the adaptation model:

\[ E(x, t) = E_b \rho(x, t)^3 \]

Here \( E(x, t) \) is the elastic modulus at location \( x \) at time point \( t \), and \( E_b \) is the elastic modulus of healthy bone tissue (5 GPa) [41-43]. \( \rho(x, t) \) is the mineralization at location \( x \) at time point \( t \), which is normalized to the average mineralization of healthy bone tissue. This means that a 10% decrease in mineralization (i.e. \( \rho(x, t) = 0.9 \)), leads to a 27% lower elastic modulus compared to healthy bone tissue ((1–0.9) × 100%). For our simulations, we assumed that the samples for which cartilage degeneration was scored as ICRS grade 0 represented healthy bone tissue. Therefore, to simulate bone adaptation to altered mineralization, the mean bone matrix mineralization measured for other OA grades was normalized to the bone matrix mineralization measured for the grade 0 samples.

For different degrees of mineralization, we performed multiple simulation series. For each series, we started with a bone structure obtained after 5000 increments of remodeling under healthy mineralization conditions. Subsequently, we changed the bone matrix mineralization in a depth-dependent manner according to the experimental data and we allowed the bone to remodel for 2500 increments. For each degree of mineralization, we performed 10 simulation series, which only differed in their random distribution of osteocytes, thereby leading to different but similar bone structures. From the resulting bone structures, we determined the bone volume fraction at different depth levels, which we compared to the experimental data. Similar to the experimentally determined parameters, bone volume fraction for the simulated structures was determined for 2 mm thick slices, of which the center was at 1, 1.5, 2, 2.5 and 3 mm distance from the cartilage.

### Results

**Cartilage degeneration according to ICRS score**

For 29 of the 53 bone–cartilage specimens all observers graded cartilage degeneration the same, for 23 specimens two observers graded cartilage degeneration the same while the third observer scored one grade higher or lower, and for 1 specimen all three observers graded cartilage degeneration differently. For this last specimen, the average of the three observers was used, while for the other specimens the grade on which at least two observers agreed was used. For each of the five ICRS grades, an example is shown in Fig. 3. In Table 3, the number of samples and the mean and standard deviation of the patients’ age at the time of surgery are shown for each ICRS grade. There were no statistically significant differences in the mean age of the patients between any of the groups.

Because we also did not find any statistically significant differences in either bone matrix mineralization or bone volume fraction between medial and lateral samples with the same degree of cartilage degeneration (Student t-test, \( p > 0.1 \)), we did not make a distinction between medial and lateral samples in our analyses. Furthermore, we decided to treat medial and lateral specimens from the same plateau as independent, based on the assumption that locally, bone matrix mineralization and bone volume fraction are more strongly related to the degree of cartilage degeneration than to patient-specific global factors. This assumption was supported by the observation that generally the degree

<table>
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<th>ICRS grade</th>
<th>No. of samples</th>
<th>Donor age (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7</td>
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</tr>
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<td>68 (8.6)</td>
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<tr>
<td>4</td>
<td>9</td>
<td>72 (15.3)</td>
</tr>
</tbody>
</table>

**Fig. 3.** ICRS grades. Samples classified according to the five ICRS cartilage degeneration grades.
of cartilage degeneration differed markedly between the two sides of one tibia plateau, together with bone matrix mineralization and bone volume fraction.

Bone matrix mineralization versus cartilage degeneration

For one patient, cartilage degeneration was graded ICRS 0 for the lateral sample and ICRS 4 for the medial sample. For these samples, CT-images and mineral distributions are shown in Fig. 4, as a typical example. Bone volume fraction is higher, while mineralization is lower in the grade 4 specimen (Fig. 4). Especially the maximum mineralization seems to be decreased compared to the grade 0 specimen. The green areas in Fig. 4b represent voxels that were partially filled with bone and partially with bone marrow. Therefore, these voxels were not taken into account for determining the mean bone matrix mineralization.

Between samples with ICRS 0, ICRS 1, ICRS 2, and ICRS 3, no statistically significant differences in bone matrix mineralization were found (Fig. 5a). In samples with ICRS 4, mean bone matrix mineralization was significantly lower compared to samples with ICRS 0 to ICRS 3 at lower depth levels. Compared to ICRS 0, this decrease varied between 6% at 1 mm distance from the cartilage, to 4% at 3 mm distance from the cartilage (Fig. 5b).

The boxplot of the slopes of the relationships between bone matrix mineralization and depth indicates that for all ICRS grades, bone matrix mineralization increased with increasing distance from the cartilage (Fig. 6). For all grades, the slope was significantly higher than zero (right-tailed Student t-test, \( p < 0.05 \)). Although the difference in bone matrix mineralization between the samples with grade 4 and with grade 0 cartilage degeneration seemed to decrease with increasing distance from the cartilage (Fig. 5b), no statistically significant difference between the slopes of any of the groups could be demonstrated (one-way ANOVA, Bonferroni post-hoc test, \( p < 0.05 \)).

Bone volume fraction versus cartilage degeneration and bone matrix mineralization

Similar to the bone matrix mineralization, for the bone volume fraction there was no difference between ICRS grades 0 to 3 (Fig. 7a–b). For ICRS grade 4, bone volume fraction was significantly increased compared to the other ICRS grades at 1 mm, 1.5 mm and 2 mm depth. Compared to grade 0, this increase varied between 69% at 1 mm distance from the cartilage and 24% at 3 mm distance from the cartilage (Fig. 7b).

After obtaining the slope of the relationship between the bone volume fraction and depth level for each sample, we grouped these slopes according the ICRS score. The boxplot of these slopes indicates that for all ICRS grades, bone volume fraction decreased with increasing distance from the cartilage (Fig. 8). For ICRS 1, 2, 3, and 4 the slope was
significantly lower than zero (left-tailed Student’s t-test, \( p < 0.05 \)). Only the slopes of the ICRS 1 and ICRS 4 groups differed significantly from each other (one-way ANOVA, Bonferroni post-hoc test, \( p < 0.05 \)). Because the ICRS 0 group contained a far outlier, and because the slopes for the ICRS 4 group were not normally distributed, we also performed Wilcoxon Signed-Rank tests to investigate whether the slopes were different from zero, and a Kruskall–Wallis test with a Bonferroni post-hoc test to compare the slopes from different groups. According to the Wilcoxon Signed-Rank test the slopes for ICRS 1, 2, 3, and 4 were significantly lower than zero (\( p < 0.05 \)), and according to the Kruskall–Wallis test, the slopes of the ICRS 0 group and ICRS 4 group differed significantly (\( p < 0.05 \)).

Bone volume fraction and bone matrix mineralization were negatively correlated (Fig. 9). When for each ICRS grade the mean values per depth level were taken (i.e. the values displayed in Figs. 5a and 7a), thereby averaging out the differences independent of OA grade and depth level, the correlation increased (Fig. 9b).

Data classified according to K–L score

For two tibia plateaus, no K–L scores were determined preoperatively, so the four samples taken from these plateaus were excluded from the analyses, leading to a total number of 49 samples (Table 4).

For both bone matrix mineralization and bone volume fraction, no statistically significant differences between any of the K–L grades were found, at any depth level (Fig. 10a–b), which may at least partly be explained by the uneven distribution of samples over the 5 K–L grades. What is interesting though, is that bone volume fraction seemed to increase gradually with increasing K–L grade (Fig. 10b), while for the ICRS score, a sudden increase was observed for grade 4 only (Fig. 7). For bone matrix mineralization this trend was not so clear, and the differences between groups were even smaller than they were for the ICRS score. This is reflected in the Pearson correlation coefficient between bone matrix mineralization (Min.BV) and bone volume fraction (BV/TV) for the mean values per depth level.

Fig. 7. Bone volume fraction. (a) Bone volume fraction (BV/TV) at different distances from the cartilage, for different grades of cartilage degeneration. (b) Bone volume fraction normalized to grade 0. *\( p < 0.05 \), **\( p < 0.01 \), one-way ANOVA, Bonferroni post-hoc test.

Fig. 8. Boxplot of the slopes of the relationships between bone volume fraction and the distance from the cartilage for each of the five ICRS grades.

Fig. 9. (a) Correlation between bone matrix mineralization (TMD) and bone volume fraction (BV/TV), taking into account all individual data points. Pearson correlation coefficient \(-0.62, p < 0.01\). (b) Correlation between bone matrix mineralization (Min.BV) and bone volume fraction (BV/TV) for the mean values per depth level for each ICRS grade. Pearson correlation coefficient \(-0.92, p < 0.01\).
for each K–L grade (Fig. 10c), which was lower than the coefficient we determined for the ICRS score (Fig. 9).

### Bone adaptation simulations

Since bone matrix mineralization did not significantly differ between grade 0 to grade 3 ICRS cartilage degeneration samples, only ICRS 0 and ICRS 4 mineralization data were used as input for the simulations. For the simulation of ICRS 4, a linear decrease in bone matrix mineralization of 6% at 1 mm depth to 4% at 3 mm depth was implemented based on the experimentally determined difference in mean bone matrix mineralization between ICRS grade 0 and ICRS grade 4 cartilage degeneration (Fig. 5b). For the ICRS 0 simulation, the bone matrix mineralization was left unchanged.

For one of the ten simulation series, the bone structures are shown in Fig. 11. Fig. 11a shows the structure used as starting point for the simulation of adaptation to the bone matrix mineralization of samples with grade 0 and grade 4 cartilage degeneration, and in Fig. 11b, the final structures for both situations are combined to highlight the differences between the bone structures. Although from Fig. 11b it can be seen that the final structures are different for the ICRS grade 0 and grade 4 simulations, no subchondral sclerosis seems to have developed in the ICRS 4 simulation.

The decrease in bone matrix mineralization for the grade 4 simulations resulted in an increase in bone volume fraction compared to the grade 0 simulations (Fig. 12). This increase varied between 9% at 1 mm depth and 4% at 3 mm depth (Fig. 12b). Although statistically significant, this increase is much less than the experimentally observed increase of 69% at 1 mm depth to 24% at 3 mm depth (Fig. 7b).

### Discussion

We aimed to investigate the relationships between cartilage degeneration, bone matrix mineralization, and bone volume fraction at a local level. With regard to the relationship between bone matrix mineralization and cartilage degeneration, we found that mineralization was lowest for the samples with the highest ICRS score. Although differences in bone matrix mineralization between the grade 4 cartilage degeneration samples and other groups were statistically significant, especially close to the cartilage, the maximum difference in mineralization with the grade 0 group was only 6%. This moderate decrease in mineralization is in concurrence with two studies from Li and Aspden [6,24], who found a decrease of 6% in bone material den-

<table>
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<th>K–L grade</th>
<th>No. of samples</th>
<th>Donor age (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3</td>
<td>65 (6.1)</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>70 (8.2)</td>
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<td>3</td>
<td>21</td>
<td>68 (11.0)</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>71 (7.1)</td>
</tr>
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</table>

Table 4
Sample data after classification according to the K–L scoring system.
sity for the subchondral plate of OA bone compared to controls and a decrease of 8% for cancellous bone, using gravimetric measurements. The decrease in mineralization that we observed with decreasing distance to the cartilage is another indication that cartilage degeneration and bone demineralization might be related, together with the observation that the difference in mineralization between the ICRS grade 4 and grade 0 samples was highest close to the cartilage. However, no statistically significant difference in the slope could be demonstrated between groups for the relationship between mineralization and depth level. While the depth-dependency of bone mineralization in OA has not been studied before in the detail as presented here, two other studies point into the same directions as our findings. In both studies, a depth-dependent difference in matrix mineralization was reported between OA and control bones, with a lower mineralization for OA bone, and the largest difference close to the cartilage [25,30].

The relationship between bone volume fraction and cartilage degeneration was opposite from the relationship between mineralization and cartilage degeneration. While bone matrix mineralization was decreased for ICRS grade 4 samples, bone volume fraction was increased for this group compared to the other ICRS grades. In addition, bone volume fraction decreased with increasing depth level, where bone mineralization increased with increasing depth level. Although bone volume fraction has been reported to decrease with increasing distance from the cartilage in bone covered by healthy cartilage as well [8,44], the slope of the relationship between bone volume fraction and depth level seemed to increase with increasing ICRS grade (Fig. 8). The 69% increase in bone volume fraction that we observed between the ICRS grade 0 and ICRS grade 4 group is comparable to differences between OA and normal bone reported in two studies on human bone tissue [7,8]. In correspondence with our data, in these studies the volume fraction of OA bone was described to decrease with increasing distance from the cartilage [7,8]. Whether the difference in bone volume fraction between OA and control bones was higher close to the cartilage, was not quantified.

As expected from their opposite relations with cartilage degeneration and depth level, bone volume fraction and bone matrix mineralization were correlated. Although the Pearson correlation coefficient of $-0.62$ indicates that only a moderate part of the variation in bone volume fraction can be explained by variations in bone matrix mineralization, the correlation was statistically significant ($p<0.01$). It is not surprising that the correlation coefficient is low, since it is known that other factors such as genetics, age, body weight, and activity level influence bone volume fraction as well. When effects not related to depth level or ICRS grade were averaged out by taking the mean values per depth level for each ICRS grade, the correlation coefficient increased to $-0.92$, indicating that 85% ($-0.92^2 \times 100\%$) of the remaining variation in bone volume fraction could be explained by variations in bone matrix mineralization. This high correlation coefficient offers supporting evidence for the hypothesis that bone demineralization could be the cause of bone sclerosis in OA. However, it does not exclude the possibility that both the decrease in mineralization and the increase in bone volume fraction were independently caused by an external factor involved in OA. Sclerosis could even indirectly be the cause of the low mineralization instead of the other way around, as the high remodeling rate associated with sclerosis might result in relatively younger and therefore less-mineralized bone tissue [18].

To obtain a better insight in the role that bone demineralization may play in the increase in bone volume fraction, we performed bone adaptation simulations. In these simulations, a depth-dependent decrease in mineralization led to a depth-dependent increase in bone volume fraction, in agreement with our experimental observations. However, despite the cubic relation between matrix mineralization and elastic...
modulus of bone tissue, the maximum increase in bone volume fraction for the grade 4 simulations compared to the grade 0 simulations was 9%, which is much less than the 69% increase that we found in the experiments. To verify that the low increase in bone volume fraction in our simulations was not a result of using 2D analyses or a high initial bone volume fraction, we also investigated the effect of decreased mineralization in 3D, for a small cube (1.35 mm × 1.35 mm × 1.35 mm) of bone tissue with a physiologic bone volume fraction (Fig. 13). In this simulation, a 5% decrease in bone matrix mineralization led to an average increase of 7% in bone volume fraction, similar to the 2D simulations. In a previously published 3D simulation study, bone adaptation in response to a tissue stiffness decrease of 20% led to an increase in bone volume fraction of approximately 10% [28], which is quite similar to our simulation results.

As the model is theoretical, it cannot prove or disprove whether the increase in bone volume fraction was caused by mechanoregulated bone adaptation in response to decreased mineralization. However, the simulations do indicate that it is highly unlikely that the 6%–4% decrease in bone demineralization can be responsible for the 69%–24% increase in bone volume fraction that we observed in the experiments. Therefore, we assume that other factors than bone demineralization, such as high joint loading or increased bone turnover contribute significantly to the development of subchondral sclerosis. This idea is supported by the differences in the changes in bone volume fraction that we observed when we classified the specimens according to ICRS and K–L scores. Locally, severe cartilage degeneration may cause additional sclerosis, while on a more global scale, bone volume fraction increases already at a relatively early stage of the disease process. An example of local sclerosis underneath an area of full-thickness cartilage erosion that we observed in one of the specimens is shown in Fig. 14. A possible explanation for this localized depth-dependent increase in bone volume could be the enhanced delivery of biochemical signals from the OA synovial fluid to the denuded subchondral bone.

A point of discussion in our study is that we treated samples from the medial and lateral side of the same joint as independent samples. Our reason for treating them as independent samples is that generally, the degree of cartilage degeneration and bone structural changes in OA differed markedly between the two sides of one tibia plateau. We considered pair-wise comparison for our study, but since we were not able to obtain samples from both sides for 11 plateaus—due to limited height of one side of these plateaus as a result of the surgical cutting plane or the presence of cysts—and because the difference in severity of OA was not the same for all plateaus, we chose to treat them as independent samples instead.

To summarize our results, we found that local changes in subchondral bone mineralization and bone volume fraction only occurred underneath severely degenerated cartilage (defined by ICRS grade 4), while at the joint scale bone volume fraction seemed to increase independent of decreased bone mineralization, and in earlier stages of the disease process (defined by low K–L grade). We did not investigate the nature of the relationships between both mineralization and bone volume fraction with cartilage degeneration, but it is possible the biochemical signaling from the degenerating cartilage or synovial fluid might be involved. This may for example explain the depth-dependency in the bone changes. Furthermore, we found that bone volume fraction and bone matrix mineralization were inversely correlated. As a possible mechanism for the relationship between these variables we considered mechanoregulated bone adaptation. Both the experimental data and the simulations indicated that a depth-dependent decrease in mineralization could cause a depth-dependent increase in bone volume fraction. However, quantitatively we showed that it can probably only explain part of the subchondral sclerosis observed in OA. Therefore, we conclude that the sclerosis in OA bone is most likely the result of the combined effect of global factors at the joint scale, and local factors at the tissue scale. Quantitatively, the global factors seem to contribute more to the changes in bone volume fraction observed in OA patients.

Acknowledgments

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References

[12] Quasnichka HL, Anderson-Mackenzie JM, Bailey AJ. Subchondral bone and liga-
ament changes precede cartilage degradation in guinea pig osteoarthritis. Bior-


Li B, 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(4):247–54.


