Treatment of Articular Cartilage Defects With Microfracture and Autologous Matrix-Induced Chondrogenesis Leads to Extensive Subchondral Bone Cyst Formation in a Sheep Model

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Background: Microfracture and the autologous matrix-induced chondrogenesis (AMIC) technique are popular for the treatment of articular cartilage defects. However, breaching of the subchondral bone plate could compromise the subchondral bone structure.

Hypothesis: Microfracture and AMIC will cause deleterious effects on the subchondral bone structure.

Study Design: Controlled laboratory study.

Methods: A total of 36 sheep received an 8-mm-diameter cartilage defect in the left medial femoral condyle. Control animals (n = 12) received no further treatment, and the rest received 5 microfracture holes either with a type I/III collagen scaffold implanted (n = 12; AMIC group) or without the collagen scaffold (n = 12; microfracture group). Macroscopic infill of defects, histology, and histomorphometry of the subchondral bone were performed at 13 and 26 weeks postoperatively, and micro–computed tomography (CT) was also performed at 26 weeks postoperatively.

Results: Microfracture and AMIC resulted in subchondral bone cyst formation in 5 of 12 (42%) and 11 of 12 (92%) specimens at 13 and 26 weeks, respectively. Subchondral bone changes induced by microfracture and AMIC were characterized by an increased percentage of bone volume, increased trabecular thickness, and a decreased trabecular separation, and extended beyond the area below the defect. High numbers of osteoclasts were observed at the cyst periphery, and all cysts communicated with the microfracture holes. Cartilage repair tissue was of poor quality and quantity at both time points and rarely reached the tidemark at 13 weeks.

Conclusion: Microfracture technique caused bone cyst formation and induced severe pathology of the subchondral bone in a sheep model.

Clinical Relevance: The potential of microfracture technique to induce subchondral bone pathology should be considered.

Keywords: microfracture; AMIC; subchondral bone pathology; subchondral bone cyst

Symptomatic articular cartilage defects are one of the most common knee injuries, arising from acute trauma, overuse, ligamentous instability, malalignment, meniscectomy, or osteochondritis dissecans. Surgical treatment options include bone marrow–stimulating techniques such as abrasion arthroplasty, cartilage resurfacing techniques and tissue engineering techniques using combinations of autologous cells, and microfracture. Currently, microfracture and the autologous matrix-induced chondrogenesis (AMIC) technique, an improved microfracture technique by implantation of a collagen scaffold with the aim of creating a biological chamber within the cartilage lesion, are popular techniques of cartilage repair by virtue of being cost-effective, one-step procedures that can be performed arthroscopically.
et al showed that microfracture achieved significant improvement in Lysholm and Tegner scores in 80% of patients in an 11-year follow-up study. A systematic review by Mithoefer et al of 28 studies and 3122 patients treated with microfracture showed an improvement in knee function. However, microfracture technique could achieve only an effective short-term outcome, and variable results after 24 months were reported.

An increasing body of evidence suggests that articular cartilage and subchondral bone are considered a single functional unit that is essential for joint function. While importance of subchondral bone integrity in the cause and surgical management of osteoarthritis has been well documented, the focus of articular cartilage repair techniques has predominantly been placed on the cartilaginous component, with very little attention to the investigation of subchondral bone integrity after cartilage repair. For example, both microfracture and AMIC techniques comprise perforation of the subchondral bone plate to facilitate hemorrhage and thereby the passage of mesenchymal stem cells, platelets, fat, and growth factors from the bone marrow into the chondral defects. Although these techniques can induce formation of functional fibrocartilage within the defect, they may lead to the damage of the subchondral bone plate. In clinical cases, an increased failure rate of autologous chondrocyte implantation has been observed after marrow stimulation techniques, as the latter has been associated with alterations in the subchondral bone.

Considering that microfracture and AMIC compromise the subchondral bone integrity in the repair of articular cartilage but little is known about their effect on the subchondral bone structure, the aim of our study was to investigate the effect of microfracture and AMIC on the subchondral bone structure in a sheep model. We hypothesized that the microfracture technique would cause subchondral bone pathology and degenerative changes.

METHODS

Animals

A total of 36 mature female sheep (Merino/Border Leicester), 5 to 7 years of age with a mean body weight (±SD) of 71.2 ± 10.6 kg, were included. The study was approved by the institutional ethical committee. The sheep were assessed to be healthy based on clinical examination, and all were free of lameness.

Surgery

Sheep were anesthetized by means of intravenous injection of ketamine (4 mg/kg) and diazepam (0.2 mg/kg) and maintained with 2% isoflurane mixed in 100% oxygen after endotracheal intubation. Additional analgesia consisted of transdermal fentanyl patches (2 μg/kg/h), epidural anesthesia (100 mg lidocaine, 0.3 mg buprenorphine), and carprofen (4 mg/kg). The left stifle joints were surgically approached via a lateral parapatellar arthrotomy as described previously. Briefly, a skin incision was made parallel and lateral to the patellar ligament, and dissection continued until the joint capsule and patellar ligament were exposed. The lateral femoropatellar ligament was transected and the patella reflected medially. The joint was visually inspected for any sign of cartilage damage or preexisting osteoarthritis. Standardized 8-mm-diameter full-thickness cartilage defects extending into the calcified cartilage layer were created on the weightbearing surface of the medial femoral condyle using a disposable skin biopsy punch and a No. 15 scalpel. The joint capsule, lateral femoropatellar ligament, fascia, subcutaneous tissue, and skin were closed in layers. Animals were housed in individual pens for 2 weeks postoperatively to restrict their movement before being turned out into small paddocks.

Treatment Groups

We evaluated 36 defects in 36 sheep at endpoints of 13 and 26 weeks. The animals were divided into 3 treatment groups as follows:

Control group (n = 12): Defects in the control group received no further treatment other than the cartilage defect.

Microfracture group (n = 12): Defects received 5 microfracture perforations, introduced using a chondro-pick, until subchondral bleeding was observed.

AMIC group (n = 12): A collagen scaffold (Celgrow type I/III porcine collagen membrane; Orthocell) was trimmed to size and implanted after the microfracture procedure, with the porous layer facing the bone surface and fixed using commercial fibrin glue (Tisseel; Baxter Healthcare Pty Ltd). The patella was then repositioned and the leg flexed 5 times. After dislocation of the patella again, the integrity of the inserted implant was confirmed by means of visual inspection.

Ex Vivo Analysis

The animals were euthanized after 13 (n = 18) or 26 weeks (n = 18) with an intravenous overdose of pentobarbitone and evaluations performed, as outlined in Figure 1. The left stifle was opened and evaluated for adverse events. Defects were photographed, and the percentage defect infill was calculated using a semi-automatic threshold-

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Histomorphometry was performed using Bioquant Osteo Histomorphometry software (Bioquant Osteo). The region of interest was drawn manually, included the subicular spongiosa immediately below the cartilage defects, and measured $8 \times 8$ mm. Subchondral bone cysts were excluded. Parameters of interest were bone volume fraction (BV/TV [percentages]), the bone surface–to–bone volume ratio as an index of structure complexity (BS/BV [in mm$^{-2}$]), trabecular thickness (Tb.Th [mm]), and trabecular separation (Tb.Sp [mm]) and were calculated automatically after semiautomatic selection of bone.

**Micro-CT.** The specimens of the 26-week groups were scanned in a micro-CT scanner (Skyscan 1172; SkyScan). As the changes of the subchondral bone at 13 weeks may still be at the fracture healing and modeling stage and may not reflect the final stage when the cartilage repair is completed, CT scanning was not performed at this time point. The micro-CT scanner was operated at a setting of 50 kV and 800 µA, with an isotropic voxel size of 20.7 µm. Images were reconstructed and analyzed using the manufacturer’s software NRecon and CTAnalyzer, respectively (SkyScan). If subchondral bone cysts were present, their borders were manually contoured and the cyst volume calculated. For evaluation of trabecular bone microarchitecture, 2 volumes of interest (VOIs) were selected based on a modified protocol described by Orth et al and were contoured manually. Standardized dimensions of 8 mm (sagittal plane) and 3.9 mm (frontal plane, 190 slices) were set for all VOIs. Total depth (apical-basal orientation) of the VOI did not exceed 8 mm. The subarticular spongiosa–defect (SAS-defect) was located strictly basally to the cartilage defect within trabecular bone with exclusion of the subchondral bone plate. The subarticular spongiosa–medial (SAS-medial) was placed in an area distant from the defect, neighboring and medial to the defect VOI. Subchondral bone cysts were excluded for the analysis of microarchitecture parameters. Semiautomated segmentation within the VOI was done using a fixed lower threshold of 89 of the maximal gray value (0-255) to accurately segment bone mineralized tissue from bone marrow. The same parameters of interest as for histomorphometry (BV/TV, BS/BV, Tb.Th, and Tb.Sp) were calculated.

The frequency of subchondral bone pathology (cyst formation, erosion of the subchondral bone plate, intrasional osteophyte formation, and upward migration of the subchondral bone plate) was evaluated.

**Statistical Analysis**

Results are expressed as mean ± SD. The macroscopic tissue infill data were not normally distributed, and therefore nonparametric analysis was performed (Kruskal Wallis test). The effect of treatment on O’Driscoll histology scores was evaluated using the Mann-Whitney U nonparametric test. Micro-CT data and histomorphometry data were normally distributed, and an independent-samples t test was performed. Correlations between macroscopic tissue infill, histological scores, micro-CT indices, and cyst size were tested via Spearman rank correlation coefficients ($\rho$).
Differences were considered significant at $P \leq .05$. All calculations were performed with SPSS (version 22.0; IBM Corp).

RESULTS

Surgery

All surgeries were performed without complications, and only 1 collagen scaffold dislodged upon flexion of the limb intraoperatively. A new scaffold was subsequently implanted. All animals recovered well from surgery. Twenty-one sheep developed seromas of the surgical sites, which resolved spontaneously within 2 weeks postoperatively, except 1 that was drained via needle aspiration. One sheep developed a left hindlimb lameness after surgery, which improved within 2 weeks.

Ex Vivo Analysis

Macroscopic Evaluation

No adverse reactions or evidence of inflammation and arthritis was present within the stifle joints and the overlying soft tissues. The macroscopic infill of the cartilage defects was incomplete and characterized by marginal repair tissue from the perimeter of the lesion toward the center for all groups and nodule-like repair tissue at the level of the microfracture holes in both treatment groups. Repair tissue in the microfracture holes at 13 weeks rarely reached above the level of the subchondral bone. One defect in the AMIC group (26 weeks) contained minimal tissue infill, and the subchondral bone was discolored with a gelatinous appearance.

At 13 weeks, the average tissue infill in the AMIC group (36% ± 8%) was not significantly different from that of the microfracture group (33% ± 5%). Both treatment groups contained a significantly higher percentage infill compared with that of the control group (20.9%; $P < .01$). The amount of tissue infill in the control group increased to 33% ± 12% at 26 weeks. The percentage tissue infill in both treatment groups nearly doubled by 26 weeks, but no significant differences existed between AMIC (68% ± 7%) and microfracture (63% ± 25%) groups. Differences between both treatment groups and the control group at 26 weeks were significant ($P \leq .02$).

Microscopic Evaluation

O’Driscoll Scoring. The results of the O’Driscoll scoring are summarized in Table 1. The median total score for the AMIC and microfracture groups 13 weeks after surgery was 7.5 (range, 3-10) and 6 (range, 3-13), respectively, and was not significantly different between groups ($P = .8$). The median total score increased to 11 (range, 6-16) and 12.5 (range, 5-20) for AMIC and microfracture, respectively, at 26 weeks. Differences between treatments were not significant ($P = .5$), but the median total scores were significantly higher at 26 weeks compared with 13 weeks ($P = .01$). Newly formed tissue at 13 weeks consisted predominantly of incompletely differentiated mesenchyme. At 26 weeks, there was a slight improvement in the cell morphology. Differences between groups or time points were not significant. The integrity of the tissue at 13 weeks was poor, although improved by 26 weeks. The thickness of the graft did not reach the level of the subchondral bone plate at 13 weeks, except for 1 sample, and rarely reached 50% of the normal cartilage thickness at 26 weeks. As the tissue at 13 weeks never reached the level of the subchondral bone plate, bonding with adjacent cartilage was nonexistent. By 26 weeks, bonding with adjacent cartilage occurred often at one end (Table 1). Most grafts were moderately hypercellular at 13 weeks and remained so over time (Table 1). The subchondral bone health was poor in all, except for 1 specimen, at both time points due to cyst formation and fibrosis. Overall, the cartilage defect was characterized by a lack of sufficient infill and tissue formation at both time points. Tissue consisted predominantly of a mixture of vascularized dense fibrous tissue at 13 weeks and progressed to more fibrocartilaginous tissue at 26 weeks. There was no evidence of cartilage formation originating from the periphery of the defect. Representative images of the

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<th>O’Driscoll Score</th>
<th>AMIC 13 Weeks</th>
<th>Microfx 13 Weeks</th>
<th>AMIC 26 Weeks</th>
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<td>6 (3-13)</td>
<td>11 (6-16)</td>
<td>12.5 (5-20)</td>
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*Data are reported as median (range). AMIC, autologous matrix-induced chondrogenesis; Microfx, microfracture.
Figure 2. Representative image of cartilage defect area treated with AMIC at 13 weeks (top: hematoxylin and eosin [H&E] stain; bottom: safranin O/fast green stain; scale bar = 2 mm). Newly formed tissue within the microfracture holes (middle and right holes) reaches to the level of the tidemark and is interrupted. Tissue within the left hole reaches around 50% of the cartilage thickness and is not bonded with the surrounding cartilage. A thin layer of undifferentiated tissue (asterisk) connects the repair tissue of the left and middle fracture hole. There is no safranin O stain uptake in the newly formed tissue in the 3 microfracture holes and reduced uptake in the surrounding cartilage. Inset 1: Repair tissue within the microfracture hole is characterized by a mixture of vascularized dense connective tissue. There is some bridging of repair tissue with the base of the surrounding cartilage but no integration more superficially, resulting in cleft formation (asterisk). Inset 2: Repair tissue within the microfracture hole is characterized by a mixture of vascularized dense connective tissue. There is no evidence of cartilage formation, and the surface is interrupted. Inset 3: Repair tissue within the microfracture hole is characterized by a mixture of vascularized dense connective tissue. There is no evidence of cartilage formation, and the surface is interrupted. Inset 4: Chondrocyte cluster formation and matrix flow are present at the periphery of the defect. A remnant of the collagen scaffold (arrow) is attached and integrated with the cartilage on the periphery of the defect. All insets: H&E stain, scale bar = 200 μm. AMIC, autologous matrix-induced chondrogenesis.
Figure 3. Representative image of cartilage defect area treated with microfracture at 26 weeks (top: safranin O/fast green stain; bottom: hematoxylin and eosin [H&E] stain; scale bar = 2 mm). Newly formed tissue remains depressed within the microfracture holes. There is more safranin O stain uptake within the repair tissue compared with tissue at 13 weeks, although differences in intensity and distribution exist within and between microfracture holes. The left microfracture hole is filled with glucosaminoglycan-rich cartilaginous tissue, although the tissue does not reach the level of the tidemark. The right hole contains a mixture of predominantly fibrous and some cartilaginous tissue. Inset 1 (safranin O/fast green stain): Repair tissue within the left microfracture hole consists of chondrocytes. Endochondral ossification is present in the deeper zone. Inset 2 (H&E stain): Repair tissue consists of predominantly fibrous tissue with debris on the surface. Inset 3: Repair tissue consists of predominantly fibrous tissue with some cartilaginous tissue in the deeper zone. A fragment of calcified cartilage is present within the debris on the surface (H&E stain). Inset 4: At the periphery of the cartilage defect, there is no sign of cartilage formation. Adhesion of the superficial cartilage layer to residual cartilage within the defect is present. All insets: scale bar = 200 μm.
surface defect area of specimens treated with the microfracture technique are shown in Figures 2 and 3.

**Qualitative Histological and Histopathological Observations**

*At 13 Weeks.* Repair tissue within the microfracture holes was highly vascular (Figure 4, inset 3), and hemorrhage was present in some samples. Reparative tissue was mainly localized in the trabecular area and rarely reached the level of the subchondral bone plate (Figure 4). No signs of inflammation or reactive foreign-body, giant-cell reactions due to the implantation of the collagen membrane were observed in the AMIC group. Mild bone marrow edema was present in the majority of the microfracture-treated specimens (Figure 4, inset 1) and was seen in half of the control specimens. Bone marrow fibrosis was also frequently present in both treatment groups and varied from mild to extensive. Subchondral bone cyst formation was present in 3 of 6 AMIC and 2 of 6 microfracture specimens (total cyst prevalence after penetration of the subchondral bone: 5/12 [42%]). Enlargement and progression of the microfracture hole deeper in the subchondral bone were noted in 2 of 6 AMIC and 2 of 6 microfracture specimens (Figure 4). In these cases, the spaces were entirely filled with graft material with minimal endochondral ossification present at the periphery (Figure 4, inset 2). The majority of subchondral bone cysts were filled with highly vascular dense fibrous tissue with high numbers of osteoclasts and resorption lacunae at the cyst periphery (Figure 4, inset 4). Trabecular changes consisted of mild sclerosis in the treatment groups, and there was minimal reversal line formation.

*At 26 Weeks.* Repair tissue within the microfracture holes that did not communicate with subchondral bone cysts and protruded above the cement line more or less had a triangular shape, with the base toward the articular surface, and contained mainly fibrocartilage. Endochondral ossification was evident at the periphery of the bone defect. When there was communication between the microfracture holes and a subchondral bone cyst, tissue within was often depressed, did not protrude above the cement line, and often consisted of undifferentiated mesenchyme toward the base and more fibrous tissue at the surface. The quantity and quality of the repair tissue over cysts appeared similar to the tissue at 13 weeks, although
more endochondral ossification was evident at the periphery of the bone defect at 26 weeks (Figure 5, inset 1), and repair tissue displayed less vasculature as compared with the 13-week samples.

Subchondral bone cysts were present in 11 of 12 samples (92%) (Figure 5). The periphery of these subchondral bone cystic lesions often consisted of fibrous tissue, often more or less organized in bundles parallel to the surrounding trabeculae (Figure 5, inset 3, and Figure 6, inset 2). High numbers of osteoclasts and resorption (Howship) lacunae were often present at the cyst periphery (Figure 5, insets 2 and 3, and Figure 6, inset 2). Cystic lesions were often filled with fibrous tissue (Figure 5, inset 4), and some contained necrotic bone fragments. Bone marrow edema, necrosis, and fibrosis were present in all treatment groups and were moderate to extensive. Mild bone marrow edema, but no necrosis or fibrosis, was present in control samples. Trabecular bone abnormalities were characterized by moderate to severe necrosis, sclerosis, and repeat bone remodeling, with frequent observation of reversal lines in all treatment samples (Figure 6, inset 1). Trabecular bone surrounding the cysts was thickened (Figures 5 and 6) and consisted of woven bone. Some trabecular sclerosis and bone remodeling were present in the control group. Osteoclastic activity was rarely observed in this group.

**Histomorphometry**

Histomorphometry results for the subarticular spongiosa immediately below the defect for condyles treated with AMIC and microfracture alone were not significantly different from each other. Therefore, both groups were combined to evaluate the effect of the microfracture technique on the subarticular spongiosa microarchitecture. The histomorphometry results are summarized in Figure 7.

**BV/TV.** The average BV/TV in the control groups at 13 and 26 weeks was similar (47% ± 7% and 43% ± 11%, respectively) (Figure 7A). Microfracture technique increased the ratio at both time points, although this difference was
significant only at 26 weeks (65% ± 8%; \( P < .001 \)). The BV/TV in the microfracture-treated group was significantly higher at 26 weeks compared with 13 weeks (\( P = .025 \)), indicating that the subchondral bone architecture progressively became denser over time.

**BS/BV.** The BS/BV ratio was not significantly altered by application of the microfracture technique, as differences between both groups were not significantly different at both time points (Figure 7B). A significant decrease in the ratio and therefore in the complexity occurred between 13 and 26 weeks in both groups. The average relative decrease was higher in the microfracture-treated group (52%) compared with the control group (38%).

**Tb.Th and Tb.Sp.** The average Tb.Th at 13 weeks for the microfracture-treated and control groups was 0.53 ± 0.24 mm and 0.57 ± 0.20 mm, respectively, and the difference was not significant. For both groups, a significant increase was noted over time (1.0 ± 0.22 mm and 0.90 ± 0.22 mm, respectively), but there was no significant difference between the microfracture-treated group and controls at 26 weeks (Figure 7C). The average Tb.Sp at both time points was significantly lower in the microfracture-treated groups compared with the control groups. Interestingly, a significant increase in Tb.Sp was seen in the control group between 13 and 26 weeks (Figure 7D). The BV/TV in the control group remained relatively constant over time,
indicating that no net bone formation or resorption occurred over time. As biomechanical forces within the epiphysis are not or minimally altered by creation of a cartilage defect without destroying the subchondral bone plate, Tb.Th may be compensated by increased Tb.Sp.

In microfracture-treated animals, an increase in relative bone volume was due to Tb.Th associated with a decrease in Tb.Sp, likely induced by altered biomechanical forces due to cyst formation and exacerbated over time.

**Micro-CT**

Representative 2- and 3-dimensional images of AMIC, microfracture, and control samples are illustrated in Figure 8. Similar to histomorphometry, micro-CT indices for the subarticular spongiosa immediately below the defect (SAS-defect) for condyles treated with AMIC and microfracture alone were not significantly different from each other, and both groups were combined to evaluate the effect of the microfracture technique on the subarticular spongiosa microarchitecture. The results for the bone morphometric parameters are summarized in Figure 9.

**BV/TV.** The mean BV/TV of the subarticular spongiosa in the control group was 55% ± 9% (Figure 9A). Microfracture technique significantly increased the BV/TV to 71% ± 10% (P = .007). The increase in BV/TV extended to a similar extent into the subarticular spongiosa distant to the defect in the microfracture-treated defects, and there were no significant differences between SAS-defect and SAS-medial values for each group.

**BS/BV.** The BS/BV ratio in the control group was 7.4 ± 1.2 mm⁻¹ immediately below and 7.6 ± 1.1 mm⁻¹ distant to the cartilage defect (Figure 9B). Microfracture treatment decreased the ratio within the spongiosa below the defect (6.2 ± 1.8 mm⁻¹), although this decrease was not significant. A significant decrease in the ratio was seen in the spongiosa distant to the cartilage defect (4.6 ± 1.5 mm⁻¹; P < .001). Similar to the BV/TV, the changes in BS/BV ratio extended beyond the area below the defect, with no significant differences between SAS-defect and SAS-medial.

**Tb.Th and Tb.Sp.** The Tb.Th within the subarticular spongiosa was 0.42 ± 0.05 mm below and 0.40 ± 0.04 mm distant to the defect. Microfracture technique increased the trabecular thickness significantly below the cartilage defect (0.53 ± 0.11 mm; P = .031) and distant to the defect (0.66 ± 0.19 mm; P = .004). Conversely, application of microfracture technique resulted in a significant decrease in Tb.Sp below and distant to the defect (Figure 9D). Changes extended to a similar extent beyond the volume below the defect.
The results of the micro-CT analysis were largely in agreement with the findings of the histomorphometry, and we can thus conclude that microfracture alters the subarticular spongiosa structure. Alterations were characterized by a significant increase in BV/TV and Tb.Th, and a significant decrease in Tb.Sp, leading to an overall less complex bone structure, mainly in the spongiosa distant to the cartilage defect. All changes in trabecular bone architecture extended beyond the volume below the affected cartilage surface.

Subchondral bone pathology was present in 100% of sheep receiving microfracture technique 26 weeks after surgery. Eleven of 12 samples (92%) treated with microfracture technique and none in the control group developed subchondral cystic lesions. The 1 sheep that did not develop a cyst developed upward migration of the subchondral bone plate in the defect and belonged to the microfracture group. The average cyst volume at 26 weeks was 119 mm$^3$ (range, 14-237 mm$^3$), and there was no significant difference between AMIC and microfracture. Erosion of the subchondral bone plate was present in 6 of 12 defects (50%) treated with microfracture technique (5 AMIC and 1 microfracture). Intraliteral osteophyte formation was observed in 2 sheep treated with AMIC and 1 control sheep (3/18 sheep; 17%), and upward migration of the subchondral bone was observed in 1 sheep treated with microfracture and 1 control sheep (2/18 sheep; 11%). There was no correlation between cyst size, macroscopic defect infill, histological O'Driscoll scores, and micro-CT indices. The lack of correlations does not further elucidate the effect of the observed subchondral bone changes on the cartilage repair tissue. However, the microscopic observation of similar quantity (depressed within microfracture hole) and quality of repair tissue at 13 weeks and 26 weeks when the hole communicated with a subchondral bone cyst supports arrested development of the repair tissue as a consequence of cyst formation and expansion.

DISCUSSION

The results of this preclinical study show that AMIC and microfracture technique facilitate repair of a surgically created articular cartilage defect more effectively than does no treatment, but induced severe pathology of the subchondral bone. Although microfracture resulted in better infill compared with no treatment, defect infill remained minimal and repair tissue was of poor quality.$^{16,24}$

While AMIC and microfracture repair the articular cartilage defects by encouraging the ingrowth of the cartilaginous tissue matrix via endochondral ossification, they also can damage the subchondral bone structures by inducing the formation of bone cysts and other subchondral bone pathology. By means of histomorphometry and micro-CT assessment, we showed that subchondral bone cysts were communicating with the surgically created microfracture holes. Histopathologic characterization of bone cysts showed typical features of inflammatory and mechanically associated changes, including osteoclastic bone resorption, dense fibrous tissue infill of cysts, and fractures through the subchondral bone plate. The associated subchondral bone pathology included an increase in the percentage bone volume, trabecular thickening, and decreased complexity of the trabecular pattern that extended beyond the region below the cartilage defect and subchondral bone cyst formation.

There are a number of other studies that have used a similar medial femoral condyle model in sheep, but their focus is generally on the repair of articular cartilage. For example, Drototka et al$^{12}$ evaluated the addition of a trilayered type I, II, and III collagen scaffold to microfractured cartilage defects and concluded that this technique did not enhance the repair at 4 and 12 months. Breinan et al$^4$ created cartilage lesions in the trochlear grooves of dogs and found an increased defect infill in AMIC using...
a type II collagen matrix compared with microfracture alone after 15 weeks. Tissue was predominantly fibrocartilaginous with less than 2% hyaline cartilage, and the authors concluded that AMIC may be beneficial for treatment of cartilage defects. We also found predominantly fibrocartilaginous tissue in this sheep model if healing was not compromised by cyst formation. Tissue connecting with subchondral bone cysts consisted mainly of undifferentiated mesenchyme and was often depressed within the microfracture hole. This indicates that cyst development compromises or at least delays cartilage repair.

Orth et al. used a similar micro-CT approach to evaluate the effect of subchondral drilling on subchondral bone microarchitecture in the medial femoral condyles of sheep and showed, similar to our study, significant alterations in bone morphometric parameters were present at the 6-month time point. However, alterations in morphometric parameters induced by microfracture technique were in the opposite direction, as Orth et al reported a decrease in BV/TV and Tb.Th and an increase in Tb.Sp compared with the defects in the study by Orth et al. and instead of microdrilling, we used a surgical awl for penetration of the subchondral bone plate. These differences could account for some variability in results.

Microfracture and the AMIC technique in the present study induced a high prevalence of subchondral bone cysts, Marchand et al. however, also observed thicker trabeculae in a rabbit model when more residual drill holes were present. Trabecular thickening possibly occurs as compensation for the failure to repair the drill holes. Micro-architectural alterations in subchondral bone comparable with that of the present study have been reported in osteoarthritic femoral heads of individuals in areas with full-thickness cartilage loss, and therefore we interpreted our findings as a degenerative change in the subchondral spongiosa rather than weakening of the microarchitecture. Conversely, Patel et al observed a decrease in BV/TV and Tb.Th and an increase in Tb.Sp in advanced osteoarthritis in human knees compared with normal knees and stated that it is possible that differences in studies of osteoarthritic trabecular bone may be due to the severity of arthrosis. The defects in our study were larger (50 mm$^2$) compared with the defects in the study by Orth et al. and instead of microdrilling, we used a surgical awl for penetration of the subchondral bone plate. These differences could account for some variability in results.

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formation (92%). In a similar preclinical model, Orth et al observed bone cyst formation 6 months after subchondral drilling in 63% of specimens, and Hoemann et al observed bone cyst formation 6 months after microfracture technique in 74% of specimens. Frisbie et al reported subchondral bone cysts in 38% of horses treated with microfracture 12 months postoperatively, as well as elevation of the subchondral bone into the defect if the calcified cartilage layer was removed before microfracturing. Another study did not report cyst formation but other subchondral bone pathology consisting of subchondral bone damage at 4 months and elevation of the subchondral bone into the defect in all specimens at 12 months in sheep. Taken together, it is clear that microfracture and the AMIC technique can induce subchondral bone pathology. The mechanism of development of bone cysts after microfracture and AMIC technique is unclear but might be due to a combination of increased synovial fluid pressure and cytokine-induced osteoclast-mediated bone resorption. Landells hypothesized that subchondral bone cysts arose from intrusion of synovial fluid into the bone at the joint surface by demonstrating communication between subchondral bone cysts and the articular surface via fissures or fractured trabeculae in osteoarthritic hip joints. This is then followed by a rim of sclerotic tissue due to displacement of trabeculae and new bone formation in response to strain. Recently, Cox et al demonstrated via a computational model that fluid pressure resulted in an irregularly shaped cavity, which became rounded and obtained a sclerotic bone rim after removal of the pressurized fluid. Li et al also reported more sclerosis of the trabecular bone surrounding bone cysts in osteoarthritic hip specimens compared with the subchondral bone distant to cysts. Although not evaluated objectively, the morphology of the cysts in the present study was irregular, supporting the role of pressured fluid in the development of these cysts. We observed abnormally higher numbers of osteoclasts and Howship lacunae at the periphery of the cystic lesions in our study. This osteoclastic bone resorption is suspected to be activated via the migration of cytokines including interleukin (IL)–1, IL-6, tumor necrosis factor alpha, and RANKL from the synovial fluid into the subchondral bone.

Surgical approach via arthrotomy, as well as the creation of an osteochondral defect and trauma to the subchondral bone, could result in inflammation with upregulation of pro-inflammatory cytokines. Cytokines in the joint fluid or within the cystic lesions were not measured in our study but would have been useful to further support this theory. Despite the striking similarities between bone cyst formation seen in advanced osteoarthritis and that seen after microfracture technique in preclinical models, the clinical significance of their presence in these animal models has been questioned. Their presence is often considered to be secondary to the immediate weightbearing of the animal after surgery. Studies that consider them “incidental” typically evaluate the quality and composition of the newly formed cartilage and find significant improvement with the “new” treatment. It is therefore plausible that these studies underestimate the influence of the subchondral bone alterations. Both translational data and clinical evidence support the importance of the subchondral bone in osteochondral healing, and we believe that future studies should include evaluation of repair of the entire osteochondral unit, not merely the articular cartilage. Similarly, Outerbridge classification on evaluation of articular cartilage damage via arthroscopy is not sufficient to grade the extent of osteochondral pathology.

There were a number of other limitations in this study. We opted to use skeletally mature sheep because we believed that they would be more representative of the patient population in need of treatment. However, mature age has been associated with a reduced osteogenic potential of stem cells, which could have contributed to the observed bone pathology/bone cyst formation. We opted to debride the cartilage defects extending into the calcified layer as this could be achieved most consistently and without inadvertently abrading the subchondral bone plate. Removing the calcified cartilage layer would expose blood vessels at the cement line, which could influence the healing within the defect. The contribution and role of the calcified layer in cartilage healing and articular cartilage repair techniques are unclear, and it remains debatable whether this layer should be removed or not. Breinan et al reported more reparative tissue with exposure of the subchondral bone, although of inferior quality compared with the more hyaline tissue formed over an intact calcified layer, and stated that an intact calcified layer is likely a critical factor. Similarly, in a horse model, Frisbie et al showed that removal of this layer increased the overall repair tissue; however, this did not result in improved histologic character, matrix proteins, or mRNA expression. In addition, removal of this layer resulted in an increase in the level of the subchondral bone into the defect space, an observation recognized in early osteoarthritis. Despite evidence of this degenerative change, the authors recommended removal of the calcified cartilage layer for debridement of clinical lesions. More recently, Hoemann et al reported that complete removal of the calcified layer in a rabbit trochlear model resulted in severe subchondral bone resorption and fibrous repair, whereas debridement into the calcified layer allowed for hyaline repair tissue formation. Similar to our study, newly formed tissue did not integrate well with the defect base when calcified cartilage was present.

Animals were allowed immediate postoperative weight-bearing, but exercise was restricted by individual housing for 2 weeks. Although implant integrity was confirmed intraoperatively, retention in the postoperative period could not be confirmed. It is widely accepted that mechanical stimulation is necessary for cartilage repair. Rodrigo et al advocated continuous passive motion after microfracture technique. Palmoski et al, however, showed that joint loading, not motion, was important to the biological and functional properties of normal articular cartilage. Recently, it has been recognized that dynamic compression and shear are necessary to induce chondrogenesis of mesenchymal stem cells. It is clear that some loading is required postoperatively, and it seems intuitive that excessive loading would be detrimental, but it remains unclear what loading is considered “excessive.” It is possible that the inability to fully control
postoperative loading and locomotion affected the severity of subchondral bone changes and, thus, that a more controlled environment may have reduced this severity. Interestingly, immobilization of the operated hindlimb for 5 days in a sheep model did not prevent subchondral bone pathology.  

Our study design did not allow further investigation of the pathogenesis of subchondral bone cyst formation and the negative effect of subchondral bone changes on the efficacy of cartilage repair.

Clinical Relevance

Bone cyst formation has been reported in up to 33% of patients and subchondral bony overgrowth in up to 49% of patients treated with microfracture. These observations after marrow-stimulating techniques have led to questioning of the usefulness of microfracture. This study in a preclinical model further confirmed that microfracture and AMIC cause subchondral bone pathology and degenerative changes. The subchondral bone cyst formation may be caused by a combination of increased synovial fluid pressure and cytokine-associated osteoclast-mediated bone resorption. Because our study showed subchondral bone alterations that were markedly similar to subchondral bone changes seen in advanced osteoarthritic joints, we speculate that microfracture technique may induce degenerative changes and contribute to the progression of osteoarthritis.

CONCLUSION

The results of this study showed that cartilage healing was significantly better for the AMIC and microfracture-treated cartilage defects compared with no treatment, but failed to show an advantage of AMIC over the microfracture technique alone. Defect infill was minimal, and newly formed tissue was of poor quality and quantity. While no inflammatory reaction caused by the collagen scaffold in the AMIC group was observed, microfracture with and without collagen scaffold led to the high occurrence of bone cyst formation in this sheep model. The formation of bone cysts may be caused by synovial fluid pressure and cytokine-associated osteoclast activation. Given the pathology of bone cysts is very similar to pathology seen in late-stage osteoarthritis, further investigation is required to confirm that microfracture technique is associated with the progression of osteoarthritis.

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