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DOI: 10.1177/0363546511403282

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Characterization of Subchondral Bone Repair for Marrow-Stimulated Chondral Defects and Its Relationship to Articular Cartilage Resurfacing

Hongmei Chen,* PhD, Anik Chevrier,* PhD, Caroline D. Hoemann,† PhD, Jun Sun,‡ MD, Wei Ouyang,* PhD, and Michael D. Buschmann,*§ PhD

Investigation performed at Ecole Polytechnique de Montreal, Montreal, Quebec, Canada

Background: Microfracture and drilling are bone marrow–stimulation techniques that initiate cartilage repair by providing access to cell populations in subchondral bone marrow. This study examined the effect of hole depth and of microfracture versus drilling on subchondral bone repair and cartilage repair in full-thickness chondral defects.

Hypotheses: Repaired subchondral bone does not reconstitute its native structure and exhibits atypical morphologic features. Drilling deeper induces greater bone remodeling and is related to improved cartilage repair.

Study Design: Controlled laboratory study.

Methods: Trochlear cartilage defects debrided of the calcified layer were prepared bilaterally in 16 skeletally mature rabbits. Drill holes were made to a depth of 2 mm or 6 mm and microfracture holes to 2 mm. Animals were sacrificed 3 months postoperatively, and joints were scanned by micro–computed tomography before histoprocessing. Bone repair was assessed with a novel scoring system and by 3-dimensional micro–computed tomography and compared with intact controls. Correlation of subchondral bone features to cartilage repair outcome was performed.

Results: Although surgical holes were partly repaired with mineralized tissue, atypical features such as residual holes, cysts, and bony overgrowth were frequently observed. For all treatment groups, repair led to an average bone volume density similar to that of the controls but the repair bone was more porous and branched as shown by significantly higher bone surface area density and connectivity density. Deeper versus shallower drilling induced a larger region of repairing and remodeling subchondral bone that positively correlated with improved cartilage repair.

Conclusion: Incomplete reconstitution of normal bone structure and continued remodeling occurred in chondral defects 3 months after bone marrow stimulation. Deep drilling induced a larger volume of repairing and remodeling bone, which appeared beneficial for chondral repair.

Clinical Relevance: Bone marrow stimulation does not reconstitute normal bone structure. Strategies that increase subchondral bone involvement in marrow stimulation could further benefit cartilage repair.

Keywords: cartilage repair; bone marrow stimulation; microfracture; drilling; subchondral bone

Although abundant research has addressed the resurfacing of articular defects and the durability of repair...
cartilage after marrow-stimulation procedures, the repair of the underlying subchondral bone has been less frequently examined. Articular cartilage and subchondral bone act as a functional osteochondral unit in the joint with each affected by alteration of the other. For example, subchondral bone reactions in cartilage repair including thickening of the bone plate, formation of intraslesional osteophytes, and subchondral cysts were observed in up to one-third of patients after MFX. Extensive surgical modification to the subchondral plate by debridement or perforation followed by immediate postoperative weight-bearing may also cause bone resorption and subchondral cyst formation. Additionally, the formation of sclerotic bone has been seen in human and animal cartilage repair models, where the increase in subchondral bone density near the original bone plate may lead to progressive cartilage degeneration and osteoarthritis. All of these studies suggest significant involvement and changes of subchondral bone may affect cartilage repair outcome.

Microfracture is a currently accepted first-line treatment for full-thickness articular cartilage defects (<4 cm²) of the knee because of its technical ease, minimal invasiveness, low cost, and demonstrated clinical benefit. Microfracture may be more widely practiced than the previously developed DRL in part because of a general belief that MFX avoids thermal damage and bone necrosis that could occur in the drilling procedure. However, we found recently, in a mature rabbit model, that by crushing and fracturing the bone, MFX left a layer of compact and necrotic bone lining the MFX holes, blocking initial access to marrow compartments. In contrast, subchondral DRL under cooled irrigation with a bur-shaped drill cleanly removed bone and bone fragments from the holes and produced no detectable acute bone necrosis. At a longer 3-month time point, we found a similar quantity and quality of cartilage repair, and higher average safranin O and collagen type II staining for 2-mm–deep DRL versus MFX but without statistical significance. A statistically significant increase in the amount and hyaline quality of repair tissue was found when drilling deeper to 6 mm versus 2 mm, which clearly increased access to bone marrow compartments. Because these different surgical techniques for placing bone marrow–stimulating holes gave rise to distinct bone fracture patterns and soft tissue repair characteristics, we were motivated here to further understand the repair of subchondral bone and its potential relationship to the quality and quantity of chondral repair in the overlying nonmineralized tissue in bone marrow stimulation. In the current study, we tested the hypotheses that bone marrow stimulation leads to altered characteristics and incomplete reconstitution of subchondral bone compared with the intact preoperative state and that the volume of subchondral bone involved in repair correlates with the quality and quantity of chondral repair tissue.

MATERIALS AND METHODS

Experimental Design

The experimental protocol was reviewed and approved by the ethics committee for animal research of the University of Montreal. A total of 20 skeletally mature (9-12 months old) female New Zealand White rabbits were used in this study, among which 16 were randomly assigned to 2 treatment groups, each comparing 2 marrow stimulation techniques bilaterally. As depicted in Figure 1A, group I animals received deep drilling (6 mm) on the left knee and shallow drilling (2 mm) on the right knee (referred to as DRL6/GrpI and DRL2/GrpII, respectively, each N = 8). The treated animals in group II had drilled holes on the left knee and microfracture holes on the right knee, both at the same depth of 2 mm (referred to as DRL2/GrpII and MFX2/GrpII, respectively, each N = 8). Four skeletally mature rabbits received no surgical intervention with a total of 8 knees serving as intact controls.

Surgical Model

After anesthesia was induced, each animal underwent sequential bilateral arthrotomies. A full-thickness cartilage defect of 4 mm × 4 mm was created using a flat surgical blade (Fine Science Tools Inc, North Vancouver, British Columbia, Canada) in the central trochlear groove with complete debridement of the calcified cartilage to expose subchondral bone with visible punctuate bleeding. We attempted to maintain an intact bone plate while completely removing the calcified cartilage. Microfracture or DRL techniques were employed to create 4 holes in each defect according to the hole pattern shown in Figure 1A. Microfracture holes of 2-mm depth and DRL holes of either 2-mm or 6-mm depth were created using customized surgical tools described in detail previously. Continuous irrigation with precooled sterile Ringer lactate solution (RLS) from a squeeze bottle was applied during drilling to minimize heating and prevent heat necrosis. The defect was also rinsed extensively with RLS to remove loose bone and cartilage debris. The patella was then repositioned and the knee closed in sutured layers. No immobilization was applied after the animals recovered from anesthesia. All operated animals received a fentanyl patch (Duragesic 25 fentanyl transdermal system, Janssen-Ortho Inc, Toronto, Ontario, Canada) for extended analgesia and were sacrificed 3 months postoperatively.

Micro-CT Scanning and Image Processing

Femoral ends were fixed in 4% paraformaldehyde/1% glutaraldehyde/0.1 M sodium cacodylate (pH 7.3) and
trimmed with a diamond saw to remove extra bone outside the defects. Fixed samples were wrapped and placed on a chamber stage with the proximal-distal axis in a vertical position and scanned by micro-CT (Skyscan X-ray Microtomography 1172, Skyscan, Kontich, Belgium) with an isotropic voxel size of 10 μm. The x-ray source voltage was 80 kV and the current was 100 μA, with a combined aluminum and copper filter to partly suppress the noise in the volume. The x-ray projections were obtained by x-ray exposure (2000 milliseconds) at 0.45°/ε intervals with a scanning angular rotation of 180°. The acquired micro-CT images were reconstructed with NRecon (version 1.6.1.2, Skyscan) with a smoothing value of 2 to reduce pixellation artefacts, and rotated in 3 dimensions using Dataviewer (version 1.4.2, Skyscan) to obtain 3 orthogonal planes (transverse, sagittal, and coronal) at the surgical site with proper orientation for subsequent image analyses. Bone tissue was segmented from marrow and soft tissue for quantitative morphometric analyses using a global thresholding procedure. Thresholding levels of gray values (95-255), confirmed by 2 operators, were used to separate bone tissue from bone marrow and soft tissue in the binary micro-CT images.

**Histology and Histomorphometry**

After micro-CT scanning, each specimen underwent histoprocessing with HCl decalcification, OCT (optimal cutting temperature) embedding, and cryosectioning. Safranin O/fast green staining and immunostaining for collagen typing were carried out using the previously published procedures. The details of histomorphometric analysis of chondral tissue repair are reported elsewhere. Briefly, the articular surface and the tidemark in the cartilage defect were identified using the flanking articular cartilage, taking into account the curvature of the trochlear groove and thickness of the adjacent cartilage. Percentage fill was then defined as the percentage of the volume of this projected defect that was filled with repair tissue.
The percentage of all nonmineralized repair tissue staining positively for safranin O (Saf-O), collagen type II (Col2), and collagen type I (Col1) was also quantified, providing a total of 4 parameters for the chondral resurfacing properties reported previously: %Fill, %Saf-O, %Col2, and %Col1.

Quantitative Bone Morphometry by 3-Dimensional Micro-CT Analysis

A 3-dimensional polygonal region of interest (ROI) was applied to the micro-CT dataset to obtain subchondral bone microarchitecture (Figure 1B and 1C). The ROI, 3 mm (width [W]) \times 3 mm (length [L]) \times 2 mm (height [H]), from the lowest point of the concave trochlear bone surface, was centered in the defect with the top surface adapted to the curvature of the projected tidemark in the defect region interpolated from the intact bone surface at distal and proximal ends outside the defects, similar to Marchand et al. The 2-mm–deep ROI contained trabecular bone and the subchondral bone plate, if any (Figure 1B and 1C). Bone overgrowth above the projected defect, if present, was excluded from the ROI. Bone morphometric parameters in the defined ROI were determined using CTAn software (version 1.9.3.0, Skyscan). The following 3-dimensional morphometric indices were calculated: bone volume fraction (BV/TV), which is the ratio of bone volume (BV) to total tissue volume (TV); bone surface density (BS/TV), the ratio of bone surface area (BS; final value with intersection surface [iS] subtracted) to TV; and connectivity density, which estimates the number of trabecular connections per cubic millimeter and thus the branching of the bone structure. These data were compared between treatments and to the control intact trochlea (Figure 1D and 1E).

Extent of Repaired and Remodeling Subchondral Bone

The area, width, and depth of repaired and remodeling subchondral bone was quantified by a reader (W.O.) blinded to treatment group using three 2-dimensional planes taken systematically from the micro-CT image dataset of each defect: midway of the defect (between the expected location of proximal and distal holes), 1 mm distal to the midplane through the expected location of the distal holes, and 1 mm proximal to the midplane through the expected location of the proximal holes. This region of repaired and remodeling subchondral bone under the cartilage defects (depicted in red in Figure 1F) was evident by its more isotropic and random trabecular pattern, with either a finer or more compact trabecular appearance that was clearly distinct compared with the intact control or the native surrounding resident bone. We used the CTAn software to quantify the region of this repaired and remodeling bone including its area, width at the bone plate (BP) region, as well as mean depth and maximum depth (Figure 1F).

Statistical Analyses

Data are expressed as mean ± standard deviation. Statistical analyses were performed with Statistica (data analysis software system, version 9.0, Statsoft Inc, Tulsa, Oklahoma). The effect of surgical procedure on scores for

### Quantitative Bone Morphometry

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#### Statistical Analyses

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**TABLE 1**

<table>
<thead>
<tr>
<th>Variables Points System for a Qualitative Assessment of Subchondral Bone Repair</th>
<th>Point Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Presence of residual holes or cyst</td>
<td>0</td>
</tr>
<tr>
<td>Subchondral cyst or large merged hole</td>
<td>0</td>
</tr>
<tr>
<td>3 or more holes</td>
<td>1</td>
</tr>
<tr>
<td>2 holes</td>
<td>2</td>
</tr>
<tr>
<td>1 hole</td>
<td>3</td>
</tr>
<tr>
<td>No visible holes</td>
<td>4</td>
</tr>
<tr>
<td>2. Bone overgrowth above projected tidemark</td>
<td>0</td>
</tr>
<tr>
<td>Proud throughout (exhibited in &gt;90% of defect width and length)</td>
<td>0</td>
</tr>
<tr>
<td>Regionally proud (exhibited in 5% to 90% of defect width and length)</td>
<td>1</td>
</tr>
<tr>
<td>Little or none (exhibited in &lt;5% of defect width and length)</td>
<td>2</td>
</tr>
<tr>
<td>3. Bone resorption</td>
<td>0</td>
</tr>
<tr>
<td>Significant</td>
<td>0</td>
</tr>
<tr>
<td>Moderate</td>
<td>1</td>
</tr>
<tr>
<td>Little or none</td>
<td>2</td>
</tr>
<tr>
<td>4. Integration to lateral adjacent bone plate</td>
<td>0</td>
</tr>
<tr>
<td>Lack of integration at 2 sides</td>
<td>0</td>
</tr>
<tr>
<td>Lack of integration at 1 side</td>
<td>1</td>
</tr>
<tr>
<td>Integration at both sides</td>
<td>2</td>
</tr>
<tr>
<td>5. Restoration of bone plate</td>
<td>0</td>
</tr>
<tr>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>Partially</td>
<td>1</td>
</tr>
<tr>
<td>Largely or completely</td>
<td>2</td>
</tr>
</tbody>
</table>

---

**Scoring System for a Qualitative Assessment of Subchondral Bone Repair**

Variables Point Scale

1. Presence of residual holes or cyst
   - Subchondral cyst or large merged hole: 0
   - 3 or more holes: 1
   - 2 holes: 2
   - 1 hole: 3
   - No visible holes: 4

2. Bone overgrowth above projected tidemark
   - Proud throughout (>90% of defect width and length): 0
   - Regionally proud (5% to 90% of defect width and length): 1
   - Little or none (<5% of defect width and length): 2

3. Bone resorption
   - Significant: 0
   - Moderate: 1
   - Little or none: 2

4. Integration to lateral adjacent bone plate
   - Lack of integration at 2 sides: 0
   - Lack of integration at 1 side: 1
   - Integration at both sides: 2

5. Restoration of bone plate
   - No: 0
   - Partially: 1
   - Largely or completely: 2

---

**Qualitative Bone Repair Assessment**

Subchondral defect repair was visually scored using micro-CT image datasets covering the entire defect region. The incidence of key subchondral bone reaction/repair phenomena including residual holes, cyst formation, bone overgrowth, bone resorption, bone integration, and bone plate restoration was recorded by visually examining the 3 orthogonal planes (transverse, sagittal, and coronal) at the surgical site in the trochlea. Their severity was assessed according to a new scoring system (Table 1) using the specified visual scaling that takes into account the frequency and extent of a set of bone repair characteristics. A score of 0 represents the worst case with regard to the severity of the particular feature and the highest score (2 or 4, depending on the category) indicates normal or close to normal bone structure (Table 1). The scores were then normalized as a percentage of the maximum possible score in each category. The assessment was done by 1 observer (H.C.) and verified by a second blinded observer (A.C.). The 2 observers attained agreement in 96% of the scores.

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**Statistical Analyses**

Data are expressed as mean ± standard deviation. Statistical analyses were performed with Statistica (data analysis software system, version 9.0, Statsoft Inc, Tulsa, Oklahoma). The effect of surgical procedure on scores for
bone repair comparing 2 different surgical procedures bilaterally was analyzed with the Wilcoxon matched pairs test. Three-dimensional morphometric parameters were analyzed for the effect of surgical procedure (DRL6/GrpI vs DRL2/GrpI and DRL2/GrpII vs MFX2/GrpII) using main-effect analysis of variance (ANOVA) with treatment and animal taken as predictors. These morphometric data were also compared with intact controls using 1-way ANOVA. The effect of surgical procedure on the extent of repaired and remodeling subchondral bone was assessed by using the general linear model with section level (through distal holes, through proximal holes, and between holes) as a repeated measure, and taking treatment and animal as predictors. The relationship between chondral holes (through distal holes, through proximal holes, and between holes) as a repeated measure, and taking treatment and animal as predictors. The relationship between chondral repair parameters (% Fill, % Saf-O, % Col2, and % Col1) and the extent of repaired and remodeling subchondral bone was examined by calculating Pearson correlation coefficients. A P value <.05 was considered statistically significant and .05 ≤ P < .2 was considered a trend.

RESULTS

General Observations and Qualitative Scoring

At 3 months postoperatively, all surgical holes were partly filled with mineralized tissue but bone repair showed several atypical structures compared with intact controls. Incomplete fill, cyst formation, bone overgrowth, and poor bone integration occurred in the defects from all treatment groups (Figure 2). We further evaluated the severity of 6 key subchondral bone features, namely the presence of residual holes, bone overgrowth, bone resorption, as well as of bone integration and bone plate repair, using a novel scoring system (Table 1), and found no significant difference in the incidence of these features comparing drilling 6 mm versus 2 mm deep in group I animals (Figure 3A). In group II, MFX2 had more residual holes than DRL2 (lower score of 25% vs 41%) but tended to produce a more intact bone plate (75% vs 25%). These effects were not statistically significant (P > .05). Other bone features were similar in the 2 treatment groups (Figure 3B). Note, bone overgrowth in the chondral region was detected in 38% to 50% of defects in the different treatment groups (Table 2). Residual holes were often detected in deeper zones of the defects (mainly in MFX2/GrpII and DRL6/GrpI), while the top of the holes could be covered by repaired bone (example in Figure 2Q).

TABLE 2

<table>
<thead>
<tr>
<th>Incidence (%)</th>
<th>DRL6/GrpI&lt;sup&gt;a&lt;/sup&gt;</th>
<th>DRL2/GrpI&lt;sup&gt;b&lt;/sup&gt;</th>
<th>DRL2/GrpII&lt;sup&gt;b&lt;/sup&gt;</th>
<th>MFX2/GrpII&lt;sup&gt;b&lt;/sup&gt;</th>
<th>All Treated (Total N = 32)</th>
<th>Clinical Data&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence (%)</td>
<td>37.5</td>
<td>50.0</td>
<td>37.5</td>
<td>50.0</td>
<td>43.8</td>
<td>25-49</td>
</tr>
</tbody>
</table>

<sup>a</sup>From group I animals (n = 8).

<sup>b</sup>From group II animals (n = 8).

<sup>c</sup>From references 3, 22, 27-29, 38

Characterization of the Extent of Repaired and Remodeling Subchondral Bone

Bone marrow stimulation induced a repair response in a subchondral region that extended beyond the original surgical perforations, consistent with cell recruitment for repair from these adjacent marrow-containing regions. Compared with normal trochlear structure (Figure 2B and 2C), the repaired and remodeling bone was less organized and more isotropic, with either a finer or a more compact trabecular appearance that was clearly distinct from the surrounding resident bone. We quantified the area, width, and depth of this repaired and remodeling subchondral bone on the micro-CT images (Figure 1F) and found no significant difference between DRL2 and MFX2 that both reached the same 2-mm depth into the subchondral bone (Figure 5B). On the other hand, drilling deeper to 6 mm versus 2 mm increased the volume of this repaired and remodeling subchondral bone region, resulting in greater cross-sectional area, as well as width and depth.
Figure 2. Repair of subchondral bone 3 months after bone marrow stimulation does not result in complete regeneration of the normal bone structure as shown by 3-dimensional visual models (left column), 2-dimensional micro-CT images (middle column), and safranin O/fast green stain (right column). A through C, normal unoperated (control). D through F, a case of good bone repair with suboptimal lateral integration (sample from DRL2/GrpII). G through I, presence of bone overgrowth (sample from DRL6/GrpI). J through L, presence of residual holes (sample from DRL2/GrpI). M through O, presence of nonmineralized connective tissue in subchondral region—a subchondral cyst (sample from MFX2/GrpII). P through R, lack of integration to adjacent bone (sample from DRL6/GrpI). Bars = 1 mm.
We correlated these parameters indicating the extent of repaired and remodeling subchondral bone with previously reported histomorphometric parameters of the soft tissue repair from all treatment groups (N = 32) regardless of type of treatment received and found moderate positive and statistically significant correlations of the extent of repaired and remodeling subchondral bone with %Fill, %Saf-O staining, and %Col2 immunostaining, and a weaker negative correlation with %Col1 immunostaining (Table 3). Results from correlation analyses for each of the 4 repair groups (data not shown) indicated that the area of repaired and remodeling subchondral bone was positively correlated with %Saf-O (P range, .002-.278; N = 8) and with %Col2 (P range, .088-.400; N=8) in all 4 treatment groups. Additionally, negative correlations were evidenced between %Col1 and the area of repaired and remodeling subchondral bone, although in most cases P > .2.

FIGURE 3. Normalized scores of key subchondral bone features in rabbit trochlear defects 3 months after receiving bone marrow stimulation. A, comparison of drilling to 6-mm deep (DRL6/GrpI) versus 2-mm deep (DRL2/GrpI) in group I. B, Comparison of drilling (DRL2/GrpII) to microfracture (MFX2/GrpII), both to the same 2-mm depth in group II. Note that a score of 100% represents normal or close to normal bone structure free from residual hole(s), bone overgrowth, and bone resorption, and with a normal bone plate and bone integration, whereas a score of 0% indicates very poor bone repair for these characteristics. Data are presented as mean ± standard deviation (N = 8). All comparisons have P > .2 comparing DRL6/GrpI to DRL2/GrpI and MFX2/GrpII to DRL2/GrpII by Wilcoxon matched pairs test, except for a trending difference (†) with P = .068.

DISCUSSION

Cartilage repair studies have usually focused on articular cartilage resurfacing but there is increasing awareness of the necessity to additionally examine subchondral bone repair.5,13,16,18,25 Bone marrow stimulation by DRL and MFX both rely on subchondral fracture repair and access to marrow cell populations to produce cartilage repair tissue, but they may have different consequences for subchondral bone and cartilage repair. Subchondral bone structure can also influence the biomechanics of the entire osteochondral unit, the repair process, and the ultimate articular cartilage resurfacing outcome. The current study focused on the subchondral mineralized bone repair at 3 months postoperatively in marrow-stimulated chondral defects and is related to our previous investigation of the articular cartilage resurfacing at the same time point.6 Our results on bone repair and the correlations between repaired and remodeling bone with articular tissue repair...
suggest a benefit in augmenting the volume of subchondral bone participating in marrow-stimulated cartilage repair to provide a higher quality and quantity of chondral repair.

Atypical structures were observed in the subchondral compartment of most defects at 3 months postoperatively (Figures 2 and 3). For example, bone overgrowth in the chondral region was observed in 38% to 50% of defects in the different treatment groups (Table 2), consistent with clinical data derived from MRI,3,22,27-29 and thus supporting the value of this mature rabbit model with a marrow-stimulated chondral defect. Although recently reported in association with MFX procedures,22,26,28 bone overgrowth occurs under many circumstances including chronic defects and/or after other cartilage repair procedures such as autologous chondrocyte implantation (ACI) and the more recent characterized chondrocyte implantation in human3,13,14,38 as well as in experimental animals undergoing ACI or marrow stimulation by DRL such as pigs,2 goats,44 and rabbits.34,40 Damage to the bone, either by trauma, excessive load, or removal of the subchondral bone plate during debridement, may provide a stimulus for endochondral ossification that can lead to hypertrophy and overgrowth.1 Alterations of joint biomechanics and joint homeostasis caused by osteochondral defects may also contribute to the generation of bone overgrowth.13,37,44 Innovative surgical techniques that prevent bone overgrowth and net loss of bone, combined with bioactive implants, may improve bone marrow stimulation procedures and provide a more consistent repair outcome.1,8,18

Our data show that bone marrow stimulation led to a bone volume fraction in the repaired bone of surgical defects that was similar to that of normal trochlea but that bone microarchitecture was markedly different (Figures 2 and 4). The repair bone was finer, more complex, branched, and connected as shown by significantly higher bone surface density (BS/TV) and higher bone connectivity density (Figure 4B and 4C). We also found that bone marrow stimulation induced a repair response in a subchondral region extending beyond the original surgical perforations (Figure 2). Drilling deeper increased the volume of repaired and remodeling subchondral bone in the defects (Figure 5A), and this increase in volume of bone participating in the repair process correlated positively to improved characteristics of cartilage repair reported previously (Table 3). These moderate and statistically significant correlations suggest increased bone activity and remodeling is beneficial for chondral resurfacing. Previous studies have shown that a higher level of bone remodeling activity is one of the main factors supporting improved cartilage repair during stimulation of the repair process using biomaterials.4,5,17,18 The presence of a remodeled and porous subchondral bone plate was also previously associated with more hyaline tissue repair and

### Table 3

Correlation of Soft Tissue Repair Parameters With Measures of the Region of Repaired and Remodeling Subchondral Bone

<table>
<thead>
<tr>
<th>Repaired and Remodeling Subchondral Bone</th>
<th>%Fill</th>
<th>%Saf-O</th>
<th>%Col2</th>
<th>%Col1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area</td>
<td>0.374 (.035)</td>
<td>0.698 (.000)</td>
<td>0.502 (.003)</td>
<td>-0.325 (.070)</td>
</tr>
<tr>
<td>Width at bone plate</td>
<td>0.457 (.009)</td>
<td>0.559 (.001)</td>
<td>0.379 (.033)</td>
<td>-0.192 (.292)</td>
</tr>
<tr>
<td>Mean depth</td>
<td>0.372 (.036)</td>
<td>0.588 (.000)</td>
<td>0.537 (.002)</td>
<td>-0.357 (.045)</td>
</tr>
<tr>
<td>Max depth</td>
<td>0.486 (.005)</td>
<td>0.486 (.005)</td>
<td>0.563 (.001)</td>
<td>-0.314 (.080)</td>
</tr>
</tbody>
</table>

*Pearson correlation coefficient R (P value) calculated from all treatment groups with N = 32. P values < .05 are shown in boldface. %Fill, percentage of the volume of the projected defect that was filled with repair tissue; %Saf-O, percentage of all nonmineralized repair tissue staining positively for safranin O; %Col2, percentage of all nonmineralized repair tissue staining positively for collagen type II; %Col1, percentage of all nonmineralized repair tissue staining positively for collagen type I.
better integration of repair cartilage with bone in rabbits.\textsuperscript{18} Bioactive scaffolds can enhance the recruitment of subchondral osteoclasts and elicit more integrated repair without inducing net bone resorption.\textsuperscript{5} Taken together, our observations and those in the literature suggest that an increased extent of subchondral bone activation and remodeling through the use of specific surgical techniques and bioactive implants can improve cartilage repair.

One important finding in the present study was that bone repair was not delayed by drilling deeper to 6 mm versus 2 mm (Figure 4). The ability to repair bone may then improve with increased access to marrow compartments and induction of a larger volume of repairing and remodeling subchondral bone produced by deeper perforations (Figure 5A). Our previously reported data on articular cartilage resurfacing in these same animals also showed superior cartilage repair quantity and quality in the 6-mm versus the 2-mm–deep drilled chondral defects after 3 months of repair.\textsuperscript{6} Thus drilling deeper may stimulate cell recruitment and subchondral remodeling, resulting in more effective production of hyaline matrix and subchondral bone repair despite greater initial bone loss in the defects. Recently, Pascarella et al\textsuperscript{8,15} introduced to their clinical practice a modified autologous matrix–induced chondrogenesis technique consisting of a lesion preparation involving drilling down to 15 mm in human condyle rather than 3- to 4-mm deep as is usual in MFX. According to the authors, this technique allowed for a maximum amount of mesenchymal stem cells to be accumulated in the defects and resulted in a good outcome for the majority of the patients up to 3 years’ follow-up.

Regarding the animal model, the rabbit knee has been widely used in experimental cartilage repair studies.\textsuperscript{4,8,18,30,40} High intra-animal variability in cartilage repair was noted by others and in our previous studies, which motivated us to use a bilateral versus unilateral animal model where treatments are compared in the same animal, and include animal as a predictor in our statistical analyses. We also use only skeletally mature animals (>9-month ages here) as younger animals spontaneously repair to a very high degree that does not represent human repair.\textsuperscript{15,46}

One limitation of the current study is that given a single intermediate time point (3 months after repair) in our study, we cannot exclude that the remodeling process of the subchondral bone in the present model is ongoing and may lead to more complete restoration of the normal bone micro-architecture after a longer time of healing. In a separate work focusing on the beneficial effect of hybrid biomaterials on rabbit chondral defect repair, we found that bone volume density in the drill-only defects (control) after 6.5-month repair was comparable with what was seen here at 3 months, but that ongoing bone remodeling processes led to further increases in bone surface density and bone connectivity density, consistent with coalescing mineralized foci as time progresses (Marchand et al, unpublished data, 2011).

CONCLUSION
Incomplete restitution of normal bone structure was observed in chondral defects at 3 months after bone marrow stimulation. Compared with shallow perforations, drilling deeper appeared to be advantageous for the repair process through increased marrow access and bone remodeling that correlated with improved cartilage resurfacing properties reported previously. Bone remodeling seen here and in published literature is a consistent positive predictor of improved chondral repair tissue quantity and quality.

ACKNOWLEDGMENT
We thank L. Dragomir, V. Lascou-Coman, and N. Tran-Khanh for their assistance in this study.

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