Bone Marrow Stimulation of the Medial Femoral Condyle Produces Inferior Cartilage and Bone Repair Compared to the Trochlea in a Rabbit Surgical Model

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ABSTRACT: The influence of the location of cartilage lesions on cartilage repair outcome is incompletely understood. This study compared cartilage and bone repair in medial femoral condylar (MFC) versus femoral trochlear (TR) defects 3 months after bone marrow stimulation in mature rabbits. Intact femurs from adult rabbits served as controls. Results from quantitative histomorphometry and histological scoring showed that bone marrow stimulation produced inferior soft tissue repair in MFC versus TR defects, as indicated by significantly lower % Fill (p = 0.03), a significant increase in collagen type I immunostaining (p < 0.00001) and lower O’Driscoll scores (p < 0.05). 3D micro-CT analysis showed that repaired TR defects regained normal un-operated values of bone volume fraction, trabecular thickness, and trabecular number, whereas in MFC defects the repaired bone architecture appeared immature and less dense compared to intact un-operated MFC controls (p < 0.0001). Severe meniscal damage was found in 28% of operated animals and was strongly correlated with (i) low cartilage defect fill, (ii) incomplete bone repair in MFC, and (iii) with a more posterior defect placement in the weight-bearing region. We conclude that the location of cartilage lesions influences cartilage repair, with better outcome in TR versus MFC defects in rabbits. Meniscal degeneration is associated with cartilage damage. © 2013 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res

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Articular cartilage lesions are a prevalent pathology found in about 65% of knee arthroscopies.1–3 The medial femoral condyle (MFC) is the most commonly affected area accounting for 34–58% of chondral injuries and is the predominant location for severe grade-IV lesions exposing subchondral bone.4,5 The trochlea (TR) as primary lesion site occurs less frequently, in 6–8% of cases,2 and is usually associated with other patellofemoral pathologies.4 Previous cartilage repair clinical research and animal studies have indicated some influence of lesion location on repair outcome,5–7 yet there are discrepancies in literature: Steadman8 reported good results in all compartments with chondral lesions after microfracture (MFX) and no association between location of lesion and functional improvement. A prospective cohort study using MFX for Outerbridge grade-III or -IV lesions of MFC, lateral femoral condyle (LFC) or TR did not reveal an effect of defect location on the outcome scores.9 In other studies, MFX was reported more successful in treating condylar versus patellofemoral lesions,10,11 producing significantly greater KOOS improvement in MFC compared to LFC defects at 3-years follow-up.12 Cartilage repair studies have been performed in animal models with defects in different anatomical locations.13–15 At 6-months of repair after MFX in sheep, more fill and better tissue integration but with a more fibrous character was reported in TR versus MFC defects.16 Less hyaline repair in TR versus MFC defects was also found in sheep after ACI.14 Thus studies to date suggest that the location of cartilage defects can exert an important influence on outcome, however no clear trend of specific locations which give rise consistently to better or worse repair have been identified. Moreover, the underlying factors which create such location dependency, for example, bone structure, the number/characteristics of bone-resident progenitors, or load bearing conditions, are also unknown.

The purpose of this study was to compare cartilage and bone repair in MFC versus TR defects after bone marrow stimulation in adult rabbits at 3 months post-operative. We also sought to determine whether different bone marrow stimulation techniques would have differential effects on defect repair in MFC. Finally the association of meniscus degeneration with cartilage repair was also investigated.

MATERIALS AND METHODS
Study Design and Rabbit Surgical Model of Bone Marrow Stimulation
The research protocol was reviewed and approved by an institutional ethics committee for animal research. Sixteen skeletally mature (9- to 10-month-old) female New Zealand White rabbits underwent sequential bilateral arthrotomies with a medial parapatellar incision. Full thickness cartilage defects were created in each knee in the central trochlear groove (TR) by manual curettage and by burring in the MFC due to higher subchondral bone density. The average dimension of TR and MFC defects were 4.0 mm × 4.5 mm and 3.7 mm × 3.9 mm, respectively, based on the available joint surface. Since the degree of flexion of the rabbit knee was not fixed at surgery, the region of the exposed condyle and the...
location of MFC defects varied along the anterior–posterior axis of the condyle and thus were quantified at sacrifice. The defects were completely debrided of the articular and calcified cartilage to expose subchondral bone with visible punctuate bleeding. Four subchondral perforations were made on each TR defect with customized surgical tools described previously.16 Two perforations were made on each MFC defect since it was too small for four holes. This hole placement resulted in 9–17% of the surface area in TR or MFC defects being perforated. Animals were randomly assigned into Group 1 (N = 8) with drill holes of 6 mm deep (DRL6/G1) in left knees and of 2 mm deep (DRL2/G1) in right knees, or Group 2 (N = 8) being drilled (DRL2/G2) in left knees and microfractured (MFX2/G2) in right knees, both to 2 mm depth. Constant irrigation with cooled sterile Ringer lactate solution was applied.16 Animals were allowed immediate ambulation in cages after recovery from anesthesia, and were sacrificed at 3 months post-operation, a time point when end stage soft tissue repair in TR defects was observed in a previous study.17 Additional four adult rabbits (12 months old) receiving no surgical intervention served as intact controls.

Gross Examination, Micro-CT Imaging and Analysis
At necropsy, joints were evaluated by two observers and photo-documented. Collected femur ends were fixed and micro-CT scanned (Skyscan x-ray microtomography 1172, Kontich, Belgium, at a 10 μm/pixel resolution). The micro-CT image stacks from MFC samples were reconstructed and repositioned as previously described for TR.18 3D micro-CT analysis was carried out by applying a region of interest (ROI) defined in Figure 1. Subchondral bone morphometric indices, including bone volume fraction (% BV/TV), bone surface density (BS/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), and connectivity density (Conn.Dn), were quantified using a global thresholding procedure in CTAn software (version 1.9.3.0, Skyscan, Kontich, Belgium).

Histoprocessing, Histology, Immunohistochemistry and Histomorphometry
Fixed samples underwent HCl decalcification and OCT embedding. Transverse sections were collected systematically from three distinct levels at the locations of the original distal and proximal holes (approximated by pictures taken at surgery), and midway between the holes in all defects, and stained with Safranin-O (Saf-O)/Fast Green, collagen type I and collagen type II. O’Driscoll histological scoring19 was carried out on blinded Saf-O stained sections using a modified scoring system,20 and the results verified by a blinded second reader. The percentage of the projected defect volume that was filled with repair tissue (% Fill) and the percentage of all nonmineralized repair tissue in defects staining positively for Safranin-O (% Saf-O), collagen type II (% Col2), and collagen type I (% Col1) were quantified (Northern Eclipse Version 8.0) as previously described.17 Intact (unoperated) specimens (N = 8 knees) were also processed to obtain the thickness of articular cartilage, the calcified cartilage layer and the subchondral bone plate from digitized images of Saf-O stained sections. The analysis was performed using NDP view software (Version 1.2.25, Hamamatsu Photonics, Hamamatsu City, Japan) with five measurements per section and three sections per specimen.

Figure 1. 3D region of interest (ROI) used in quantitative micro-CT analysis, defined as 3 mm (W) × 3 mm (L) × 2 mm (H) with its top surface coinciding with the bone surface in intact controls (A and B), and the projected bone surface in defects (C and D). Arrows indicate peripheral osteophyte formation.
Scoring of Meniscus Degeneration and Osteophytes

The severity of medial meniscal damage was graded (Fig. 2A–F). A score of five represented a healthy meniscus, whereas a score of 0 indicates a severely degenerated, fragmented meniscus. Peripheral osteophyte formation were encoded as 0 if present at both the medial and lateral sides of the joints, 1 if present at the medial side only, and 2 if no osteophyte was observed. The placement of MFC cartilage defects was characterized by two investigators on photographs taken at necropsy, and rated as 4, anterior (e.g., Fig. 2G); 3, mid-anterior; 2, mid; 1, mid-posterior; and 0, posterior (e.g., Fig. 2H), according to the ICRS grid map of the knee. The higher the score, the more anterior was the initially created defect on the MFC.

Statistical Analysis

Statistical analyses were performed with Statistica (version 10.0, Statsoft, Inc., Tulsa, OK). Numerical data were presented as mean ± standard deviation. The effect of defect location (TR vs. MFC) was analyzed using the General Linear Model (GLM) with location and animal taken as predictors. The effects of treatment (DRL6 vs. DRL2 and DRL2 vs. MFX2) on soft tissue repair and subchondral bone repair in MFC defects were evaluated by GLM with treat-

Figure 2. Representative photographs showing levels (0–5) of damaged medial menisci at 3 months after bone marrow stimulation on cartilage defects in the MFC (A–F). Arrows show medial menisci that were normal (score 5 in A), intact but slightly rough surface (score 4 in B), intact with fibrillated and rough surface (score 3 in C), split (score 2 in D), largely damaged (score 1 in E), and completely fragmented (score 0 in F). G and H are photographs taken at necropsy to show different anterior versus posterior placement of the defect on MFC.
ment and animal taken as predictors. Histomorphometric parameters of % Fill, % Saf-O, % Col2, and % Col1 were analyzed together as an aggregate indicator of overall quantity and quality of repair cartilage by GLM with two repeated-measure variables—the histomorphometric parameter and the section level (through distal holes, through proximal holes and between holes). The subchondral bone morphometric data from treated defects were compared to those from intact controls using one-way ANOVA. O’Driscoll histological scores in TR and MFC defects were compared with the nonparametric Wilcoxon matched pairs test, as was the effect of bone marrow stimulation techniques on meniscus scores. Correlations between meniscal integrity with defect repair features including % Fill, O’Driscoll sum score, and % BV/TV, the presence of osteophytes, and the anterior versus posterior placement of MFC defects were analyzed by calculating the Pearson correlation coefficients \( r \). \( p \) values < 0.05 were considered statistically significant.

RESULTS

Intact TR and MFC From Un-Operated Rabbits Had Distinct Articular Cartilage and Subchondral Bone Structure

We analyzed TR and MFC from intact skeletally mature rabbit knees by histomorphometry and micro-CT, and found structural differences (Fig. 3). The articular cartilage layer was two-fold thicker in MFC than in TR (252 ± 58 µm vs. 129 ± 33 µm), as was the calcified cartilage layer (120 ± 34 µm vs. 67 ± 30 µm). The subchondral bone plate was also thicker in MFC versus TR (383 ± 157 µm vs. 259 ± 161 µm. Fig. 3G). The epiphyseal line in rabbit TR was about 3–4 mm from the articular surface such that our 6 mm deep drill holes penetrated through it and reached the metaphyseal bone marrow (Fig. 3D) 16; in contrast, it was inaccessible by deep perforation in the MFC (Fig. 3A). Quantitative micro-CT analysis on the 3D ROIs (Fig. 1A and B) showed significant differences between intact TR and MFC. The subchondral bone density was higher and the trabeculae were thicker, less numerous, and less connected in MFC versus TR (\( p < 0.05 \), Fig. 4).

Bone Marrow Stimulation Produced Inferior Cartilage Repair in MFC Versus TR Defects

At 3 months post-operative, MFC defects showed lower quality repair compared to TR (Figs. 5 and 6). Six out of 32 MFC defects had depressed repair tissue with very little fill (e.g., Figs. 5E, 6I and 6K) while no TR defects were depressed. Quantitative histomorphometry revealed a significant decrease in % Fill in the chondral zone of MFC versus TR defects (\( p = 0.031 \), Fig. 7), consistent with lower O’Driscoll scores in MFC for repair tissue thickness (\( p = 0.026 \), data not shown). Both MFC and TR defects were equally positive for Col2 staining in the repair tissue matrix (% Col2 ≥ 80%, Fig. 7). However, Col1 was more widespread in MFC versus TR defects (% Col1: 51.9% vs. 13.4%, \( p < 0.00001 \)) (Fig. 7). Improvement in tissue repair quality in TR versus MFC was significant (\( p = 0.002 \)) when the four parameters (% Fill, % Saf-O, % Col2, and % Col1 in Fig. 7) were analyzed together as repeated-measure variables for an aggregate indicator of overall repair quantity and quality. The O’Driscoll sum score was significantly lower in MFC versus TR defects (\( p = 0.004 \)), as were the subcategories of Saf-O staining of the matrix, absence of hypocellularity and of chondrocyte clustering, as well as adjacent articular cartilage health (\( p < 0.05 \), data not shown). The different surgical treatments (DRL6 vs. DRL2 and DRL2 vs. MFX2) did not produce different cartilage repair outcomes in the MFC (data not shown) unlike the improvement seen with deeper drilling (6 mm vs. 2 mm) published previously for TR. 17

Figure 3. Structure of MFC (A–C) and TR (D–F) in intact rabbits, and thickness of articular cartilage (AC), calcified cartilage (CC) and subchondral bone plate (BP) in MFC and TR (G). Arrows show the epiphyseal line in TR, below which the metaphyseal bone marrow (*) is accessible by 6 mm deep perforations (indicated by the red dash lines).
Figure 4. Subchondral bone structure by 3D micro-CT in the ROIs of Figure 1. Significant differences were observed comparing all five subchondral bone parameters (A–E) in MFC defects to intact MFC (*p < 0.00008, one-way ANOVA). In contrast, TR repair bone had % BV/TV and average trabecular thickness (Tb.Th) similar to those from intact TR, but higher BS/TV and connectivity density (Conn.Dn) (p < 0.006). Significant differences (p < 0.05) were found in all bone morphometric indices comparing intact TR to intact MFC, except for % BV/TV. †p < 0.05 comparing intact TR to intact MFC controls.

Figure 5. Representative Safranin-O/Fast Green staining of cartilage defects from Group 1 animals, treated with 6 mm deep drill (DRL6/G1, left panel) in left knees or 2 mm shallow drill (DRL2/G2, right panel) in right knees, showing the best (A–D), median (E–H), and worst (I–L) cases of 3 months tissue repair in MFC (A, E, I, C, G, & K) and TR (B, F, J, D, H, & L) according to the sum O’Driscoll score. Bar = 1 mm.
Bone Marrow Stimulation Led to Worse Subchondral Bone Repair in MFC Versus TR Defects

The repair of subchondral bone was still on-going at 3 months post-operative, and bone structure underneath the defects displayed clear differences compared to intact controls. Quantitative micro-CT analysis revealed that subchondral bone in MFC defects had a significantly lower bone volume fraction than that of intact MFC controls (45.8% vs. 63.4%, \( p < 0.00004 \)). Fig. 4A). Additional bone morphometric features in MFC defects were significantly different from those in intact MFC, with thinner but more numerous trabeculae, and with greater bone surface density and connectivity density (Fig. 4B–E). In contrast, the subchondral bone volume density was restored in TR defects, with similar trabecular thickness and trabecular number values as in intact TR (\( p > 0.2 \)), although the repaired bone still had a higher level of remodeling and connectivity compared to the native TR (Fig. 4B and E).

Medial Meniscus Degeneration Was Correlated to a More Posterior Placement of Cartilage Defects and to Reduced Quality of Repair in the MFC

Damage to the medial meniscus was found in 53% of all operated knees at necropsy while the lateral meniscus, patella and tibial plateau were macroscopically normal. Most meniscal damage was minor (roughened surface, score \( \geq 3 \)) with meniscal integrity upheld in most knees (e.g., Fig. 2A–C). But 9 out of 32 (28%) menisci showed severe degeneration (Fig. 2D–F) and four were completely fragmented. No association was detected between meniscus damage and the specific bone marrow stimulation technique (DRL6, DRL2, or MFX2). However, meniscus damage was significantly correlated with lower % Fill, lower O’Driscoll sum score, and lower % BV/TV in MFC defects (Table 1). Peripheral osteophyte formation...
MFC Defects, and Peripheral Osteophyte Formation

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operative. We found less fill in MFC versus TR defects,
consistent with the previous finding in sheep after
MFX15; yet in contrast to this sheep model, we found a
more fibrous character of the repair tissue in MFC in
rabbits. The location dependency seen in different
species or between human and animals suggested that
some underlying factors such as bone structure, the
number or characteristics of bone-resident progenitors,
and load bearing conditions may be responsible for
location dependent outcomes. Unlike the convex MFC,
TR could provide a shielding effect for the fibrin blood
clot and initial granulation repair tissue in the
debrided lesion, which has been shown to be a central
facet to subsequent defect repair.15,21 Since the relative
compressive versus shear loading influences the
nature of the repair, the articular conformity of
tibiofemoral and patellofemoral compartment affects
contact mechanics,22 and the MFC is weight-bearing
while TR is partly weight-bearing in rabbits,23 the
observed degenerative changes including loss of gly-
cosaminoglycan, hypocellularity and cell clustering at
the rims of MFC defects and fibrillation in the repair
matrix may be related to unrestricted ambulation
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ics.

We previously reported that deeper drilling im-
proved repair compared to shallower drilling in TR
defects17,18; this effect, however, was not seen here for
MFC defects. This could be related to inherent struc-
tural differences in TR versus MFC where our 6 mm
deep drill holes reached the metaphyseal bone marrow
in TR,16 but not in MFC (Fig. 3A and D). It was
previously suggested that different cell types may
reside in specific regions of the marrow,24 and deep
drilling in TR may potentially recruit a greater
number of cells and a variety of cell types from the
deep marrow stroma, resulting in improved cartilage
repair.17 Nonetheless, the superiority of TR versus
MFC repair was not solely due to access to deep
marrow stroma, since significant effects of defect
location were always detected on aggregate histo-
morphometry features, % Col1 and % BV/TV even
when all eight deep drill samples were excluded from
the statistical analysis.

Improved repair in TR versus MFC may also be due
to a greater chondrogenic potential of progenitor cells
in subchondral bone for TR versus MFC, and is
consistent with our observations in a related study of
greater chondrogenesis occurring in TR versus MFC
defects at 3 weeks post-operative in mature rabbits.25
Additionally, there may be other features that poten-
tially affect the repair in MFC versus TR, including
subchondral vascularization,21 remodeling,20,26 local
inflammation, debridement of a curved condylar
versus concave trochlear surface, and biomechanical
factors including damaged menisci.

We found that meniscus degeneration seen in some
rabbits after marrow stimulation (Fig. 2) was correlat-
ed to poor cartilage repair and to posterior placement
of the MFC defects (Table 1). To our knowledge,
meniscus degeneration has not been reported in an
animal model for cartilage repair, although medial
meniscus tears are known as one of the most common
pathologies concomitant with articular lesions in
humans.1–3 The defects placed more posteriorly in
MFC directly oppose the medical meniscus, possibly
causing meniscus damage by contact force with the
defect. In this study, we used a rather aggressive
model, with defects created bilaterally and concurrent-
ly in TR and MFC (four defects per animal), and the
defects occupied a large portion of the surface area in
rabbit knees, about 65% width and 25% surface area,
potentially promoting osteophyte formation at joint
margins, especially at the medial site of joint loading
(Fig. 1C and D).

In conclusion, our study revealed that cartilage
repair by bone marrow stimulation depends on the
location of the cartilage defect whether placed on TR
versus MFC and more specifically anterior versus
posterior on the MFC. The underlying factors involved
in this differential repair outcome that require further
study include subchondral bone structure, chondro-
genic potential of trabecular bone-resident precursors,
as well as geometric and biomechanical factors.

Table 1. Pearson Correlation Coefficients (r) of Meniscal Integrity With Repair Features and Anterior Placement of MFC Defects, and Peripheral Osteophyte Formation

<table>
<thead>
<tr>
<th>% Fill</th>
<th>Sum O’Driscoll Score</th>
<th>% BV/TV</th>
<th>Anterior MFC Defect Placement</th>
<th>Absence of Osteophytes</th>
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<tbody>
<tr>
<td>r (p-value)</td>
<td>0.504 (0.003)</td>
<td>0.587 (&lt;0.001)</td>
<td>0.403 (0.025)</td>
<td>0.650 (&lt;0.001)</td>
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REFERENCES