Depth of Subchondral Perforation Influences the Outcome of Bone Marrow Stimulation Cartilage Repair

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ABSTRACT: Subchondral drilling and microfracture are bone marrow stimulation techniques commonly used for the treatment of cartilage defects. Few studies to date have examined the technical variants which may influence the success of the cartilage repair procedures. This study compared the effect of hole depth (6 mm vs. 2 mm) and hole type (drill vs. microfracture) on chondral defect repair using a mature rabbit model. Results from quantitative histomorphometry and histological scoring showed that deeper versus shallower drilling elicited a greater fill of the cartilage defect with a more hyaline character in the repair matrix indicated by significant improvement (p = 0.021) in the aggregate measure of increased cartilage defect fill, increased glycosaminoglycan and type II collagen content and reduced type I collagen content of total soft repair tissue. Compared to microfracture at the same 2 mm depth, drilling to 2 mm produced a similar quantity and quality of cartilage repair (p = 0.120) according to the aggregate indicator described above. We conclude that the depth of bone marrow stimulation can exert important influences on cartilage repair outcomes. © 2011 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 29:1178–1184, 2011

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Articular cartilage defects are a common pathology1 that if left untreated, can progress to osteoarthritis resulting in significant musculoskeletal morbidity. The poor intrinsic healing capacity of articular cartilage is partly the result of its avascular nature, which abrogates wound repair processes at the site of cartilage injury, along with the limited ability of resident chondrocytes to effect tissue repair.2,3 Bone marrow stimulation, a widely practiced treatment option, introduces perforations in the subchondral bone and initiates bleeding, thereby exposing the debrided cartilage lesion to the underlying bone marrow stroma which contains a repository of pluripotential cells capable of repairing soft and hard tissues.4 Providing access channels to marrow stroma and allowing cell migration into the articular defect are essential features of marrow stimulation-based cartilage repair.

Bone marrow stimulation can be achieved by abrasion arthroplasty,5 subchondral drilling (DRL),6 and microfracture (MFX).7 Subchondral DRL introduced by Pridie in 19599 was prevalent as a treatment for various osteochondral defects for decades.5,8–10 Presently, Steadman’s MFX approach7 has become a first-line treatment option for full-thickness articular cartilage defects of the knee with significant symptomatic relief and improvement of knee function, especially for the first 2 years postoperative.11 Previous marrow stimulation studies have demonstrated the importance of controlling defect debridement, integrity of subchondral bone plate, and postoperative rehabilitation.12,13 However, no clinical and animal studies specifically controlled the perforation depth, nor was its effect on cartilage repair outcomes examined. Additionally, direct comparison of MFX to DRL has not been performed to date.

It has been stated that by using an awl, MFX avoids thermal damage to subchondral bone, thus producing less bone necrosis and is therefore potentially superior to Pridie drilling which employs a hand-driven or motorized drill.7,13 Nonetheless, results from our recent study suggest that microfracture is not superior to drilling in terms of reducing initial bone necrosis.14 We also found that in acute defects, holes drilled deeper to 6 mm versus 2 mm provided greater access to marrow compartments. The objective of this study was to examine the influence of these surgical options on mid-term cartilage repair outcome. We tested the specific hypotheses that deep DRL generates repair tissue of a greater quality and higher quantity than shallow DRL and that DRL improves cartilage repair compared to MFX, the latter by removing dead bone rather than compacting it around the periphery of the hole as we recently found.14 We sought to establish relationships between features of acute perforations and defect healing at 3 months in rabbits, with the ultimate goal of improved understanding and optimization of marrow stimulation techniques.

MATERIALS AND METHODS
Experimental Design and Surgical Procedure
The research protocol was reviewed and approved by an institutional ethics committee for animal research. Sixteen skeletally mature (9–10 months old) female New Zealand White rabbits were randomly assigned to two groups, each (N = 8) comparing two marrow stimulation techniques bilaterally. As depicted in Figure 1A, Group I received deep drilling (6 mm) on the left knees and shallow drilling (2 mm) on
chondral bone with visible punctuate bleeding since calcified complete debridement of the calcified cartilage to expose sub-

Vancouver, Canada) in the central trochlear groove with using a flat surgical blade (Fine Science Tools, Inc., North artrotomies. A cartilage defect (4 mm respectively). Each animal underwent sequential bilateral depth of 2 mm (referred to as DRL2/GrpII and MFX2/GrpII, holes both at 2 mm deep. (B) Schematics of tissue repair regions

to shallow drill holes (DRL2/GrpII, 6 mm) to shallow drill holes (DRL2/GrpI, 2 mm), and Group II compares MFX2/GrpII to DRL2/GrpII holes. (Fig. 1A) by using miniature DRL bits (burr diameter 0.9 mm) that permitted depth control of DRL holes to either 6 or 2 mm, or by using a custom-machined MFX awl with a mallet that pierced conically shaped holes 2 mm deep with a 1 mm base diameter.14 Cylindrical DRL holes were made using a high-speed microdrill (Fine Science Tools, Inc.) under continuous irrigation with precooled sterile Ringer's lactate solution (RLS) from a squeeze bottle, which limits thermal necrosis.14 After drilling, 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. The knees were allowed unrestricted motion after the animals recovered from anesthesia. All animals received a fentanyl transdermal patch (Duragesic 25, Jansen-Ortho, Inc., Toronto, Canada) for extended analgesia, and were sacrificed 3 months postoperatively.

the right (referred to as DRL6/GrpI and DRL2/GrpI, respectively) while Group II animals had DRL holes on the left knees and MFX holes on the right knee, both at the same depth of 2 mm (referred to as DRL2/GrpII and MFX2/GrpII, respectively). Each animal underwent sequential bilateral arthrotemies. A cartilage defect (4 mm × 4 mm) was created using a flat surgical blade (Fine Science Tools, Inc., North Vancouver, Canada) in the central trochlear groove with complete debridement of the calcified cartilage to expose subchondral bone with visible punctuate bleeding since calcified cartilage is known to block repair.15 Effort was made to retain an intact bone plate as much as possible. DRL or MFX techniques were employed to create four holes in each defect (Fig. 1A) by using miniature DRL bits (burr diameter 0.9 mm) that permitted depth control of DRL holes to either 6 or 2 mm, or by using a custom-machined MFX awl with a mallet that pierced conically shaped holes 2 mm deep with a 1 mm base diameter.14 Cylindrical DRL holes were made using a high-speed microdrill (Fine Science Tools, Inc.) under continuous irrigation with precooled sterile Ringer's lactate solution (RLS) from a squeeze bottle, which limits thermal necrosis.14 After drilling, 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. The knees were allowed unrestricted motion after the animals recovered from anesthesia. All animals received a fentanyl transdermal patch (Duragesic 25, Jansen-Ortho, Inc., Toronto, Canada) for extended analgesia, and were sacrificed 3 months postoperatively.

Histoprocessing, Histostaining, and Immunohistochemistry

Collected femur ends were fixed in 4% paraformaldehyde/1% glutaraldehyde/0.1 M sodium cacodylate (pH 7.3), decalcified in HCl and OCT-embedded. Transverse sections were collected systematically from three levels at the locations of the original distal and proximal holes, and midway between the holes in all defects. Safranin-O (Safo-O) Fast Green staining and immunostaining for collagen type I (Col1) and collagen type II (Col2) were performed as previously described.16

Histological Scoring and Quantitative Histomorphometry

All analyses were carried out on three sections per defect collected from the aforementioned three levels. O'Driscoll histological scoring17 was performed by two independent blinded observers on Safo-O-stained sections. Quantitative histomorphometry on Safo-O-stained sections was performed using Northern Eclipse software (V8.0, Empix Imaging, Inc., Mississauga, ON, Canada). The total region of soft tissue repair was defined and measured as total soft tissue volume (orange polygon in Fig. 1B). Percent Safo-O+ repair tissue in total soft tissue volume was obtained by Northern Eclipse threshold analysis. The projected cartilage defect (red lines Fig. 1B) was established from the flanking articular cartilage, taking into account the curvature of the trochlear groove and thickness of the adjacent cartilage. %Fill was defined as percent tissue repair volume within the projected defect based on 2D cross-sectional areas of the projected defect area in histology sections from three distinct sites in each defect. Basal attachment of soft repair tissue was quantified with Northern Eclipse18 as percent attached over the total length of the interface between soft repair tissue and subchondral bone. Percent tissue volume positive for collagen type I and for collagen type II in the total soft tissue volume was quantified using Bioquant Osteo II software (V8.0, Bioquant Image Analysis Corp., Nashville, TN).

Statistical Analysis

Statistical analyses were performed with repeated measures in the Generalized Linear Model, Statistica (version 9.0, Statsoft, Inc., Tulsa, OK). Differences in histomorphometric parameters were compared between surgical treatments (DRL6 vs. DRL2 and DRL2 vs. MFX2) with treatment and animal taken as predictors, thus benefiting from the bilateral design where two treatments are compared in the same animal, thus reducing the effect of inter-animal variability. %Fill, %Safo-O, %Col1, and %Col2 were also analyzed together as an aggregate indicator of overall quantity and quality of repair cartilage by specifying two repeated-measure variables—the histomorphometric parameter and the section number (1–3). O'Driscoll scores were similarly analyzed with each of the three sections scored by two blinded observers constituting six repeated measures. Spearman rank order correlations between histomorphometric parameters were computed. ^p < 0.05 was considered statistically significant.

RESULTS

General Observations

After 3 months of repair, Safo-O staining of proteoglycan-rich repair tissue was detected mostly in the deep-mid region of repair tissue (Figs. 2A–C and 3A,C,D). Immuno-reactive Col2 was more widespread in the repair matrix, covering a larger area than Safo-O stain
in all treatment groups (compare E–H to A–D in Figs. 2 and 3). The superficial repair tissue in most specimens (81%) contained Col1 and had diminished intensity or depleted Saf-O stain indicating a component of fibrocartilaginous repair although three specimens were uniformly stained with Col2 with no Col1 and possessed quite hyaline matrix characteristics (example in Fig. 2G,K). The tidemark and zonal organization of articular cartilage were not yet re-established at the 3-month time point. Repair tissues frequently failed to bond with adjacent cartilage (Figs. 2A–D and 3A–D), as reflected by low O’Driscoll bonding scores (<1) for all treatment groups. Percent basal attachment of the repair tissue to the underlying bone was ~80% with no significant differences among treatments.

**Figure 2.** Safranin-O/Fast Green staining (A–D), collagen type II (E–H), and collagen type I (I–L) immunostaining in Group I comparing deep 6 mm (DRL6) to shallow 2 mm (DRL2) drilling, after 3 months of repair. The images shown in the top (A–B, E–F, and I–J) panel were taken from representative sections from bilateral defects in the same animal, whereas those in the middle panel (C–D, G–H, and K–L) were from another animal in the same group. M: histomorphometric analyses of soft tissue repair. *Significant effect ($p = 0.015$, $N = 8$) for DRL6 versus DRL2. Improvement in tissue repair due to deep compared to shallow drilling was significant ($p = 0.021$) when the four parameters (%Fill, %Saf-O, %Col2, and %Col1) were analyzed together as repeated-measure variables for an aggregate indicator of overall repair quantity and quality.
Deeper Drilling Produced Statistically Superior Cartilage Repair than Shallower Drilling

Quantitative histomorphometry revealed that compared to shallow DRL2, deep DRL6 produced 85.6% versus 65.3% of %Fill ($p = 0.015$), 41.1% versus 29.9% of %Saf O ($p = 0.125$), 80.0% versus 65.6% of %Col2 ($p = 0.094$), and 12.3% versus 20.7% of %Col1 ($p = 0.251$; Fig. 2M), with only %Fill being statistically significant at this small sample size ($N = 8$). The trending of increased Saf-O stain for DRL6 versus DRL2 was corroborated by O'Driscoll matrix stain scoring ($p = 0.106$). Improvement in tissue repair...
due to deep versus shallow drilling was significant ($p = 0.021$) when four parameters (%Fill, %Saf-O, %Col2, and %Col1) were analyzed together as repeated-measure variables as an aggregate indicator of overall repair quantity and quality. Additionally, %Fill was positively correlated with %Saf-O and %Col2, and negatively correlated with %Col1. These weak ($r^2 = 0.2–0.3$) but statistically significant ($p < 0.05$) correlations suggested that these correlated hyaline matrix characteristics are linked and can be improved together by specific surgical techniques.

**Drilling Did Not Statistically Improve Cartilage Repair Compared to Microfracture**

Our data showed that compared to MFX2, DRL2 elicited 56% more total soft repair tissue ($p = 0.176$, orange polygon in Fig. 1B, data not shown); within this soft repair tissue matrix DRL2 elicited a 55% increase in %Saf-O ($p = 0.223$), a higher %Col2 ($p = 0.109$), and similar %Col1 (~10%) (Fig. 3M) compared to MFX2. These differences were not statistically significant. %Fill within the projected cartilage defect (red lines, Fig. 1B) was similar in both DRL2 and MFX2 repair. Improvement in tissue repair due to DRL compared to MFX was also not statistically significant ($p = 0.120$) when these four parameters were analyzed together as repeated-measure variables for an aggregate indicator of overall repair quantity and quality, consistent with O'Driscoll scoring results. Correlation analysis revealed that %Saf-O in MFX2 repair was positively correlated with total soft tissue volume and negatively correlated with %Col1; the latter correlation was also seen in DRL2 repair. These correlations were statistically significant but weak ($p < 0.05$, $r^2 = 0.2–0.5$). Additionally, %Fill and %Col2 appeared to be higher for DRL2 repair in Group II than in Group I (compare Figs. 2M to 3M). However, these differences are difficult to interpret due to high inter-animal variability and the fact that a bilateral study, we used burr-shaped drills which removed bone debris effectively, along with continuous cool irrigation through it while the shallow holes did not. It has been previously suggested that successful wound healing and cartilage repair relied on increased recruitment of bone marrow-derived mesenchymal cells and that different cell types may reside in specific regions of the marrow. In this study, deep DRL created more access channels to the marrow and may potentially recruit a greater number of cells and a variety of cell types from the deep marrow stroma, resulting in improved cartilage repair (Fig. 2). We believe that the statistically significant one-third increase in %Fill detected in the deeper DRL6 versus the shallower DRL2 defects is functionally important in terms of the ability of the repair tissue to bear load, since many clinical marrow stimulation studies only show partial fill of the cartilage lesion that is most likely not effectively load-bearing. The higher %Col2 and %GAG and lower %Col1 observed in the DRL6 defects relative to DRL2 defects (Fig. 2) may also render the repair tissue more durable and able to bear load.

There is abundant research addressing the concern of thermal damage of bone due to drilling. Often described is the use of Kirschner-wires, which have a smooth surface without flutes and may then compress bone debris without removal, leading to increased bone density and increased heat due to friction. Of note, while a variety of clinically available drill designs with finely tuned parameters have been developed to reduce heat generation, the older drilling technique using Kirschner-wires is still practiced clinically. In our study, we used burr-shaped drills which removed bone debris effectively, along with continuous cool irrigation to minimize heating. These aspects help limit necrosis and preserve the vitality of surrounding bone according to our previous study, creating a favorable local environment, without apparent thermal necrosis, to attract reparative marrow cells for enhanced cartilage repair.

Although our collective data did not show significant improvement in defect repair comparing DRL to MFX at the same 2 mm depth, with a sample size of 8 and $p = 0.120$ for overall repair quantity and quality, repair matrix with hyaline-like or mixed hyaline/fibrocartilage characteristics was observed in many drilled defects (Figs. 2 and 3). Shapiro et al. also reported hyaline-like repair following drilling with good lateral integration and complete restoration of

Bone marrow stimulation-based cartilage repair relies on recruitment of marrow-derived mesenchymal progenitor cells to heal the defects. Providing access channels to marrow stroma is thus a prerequisite for these procedures. The improved repair observed here in deeper drilling may result from increased access to marrow compartments where the 6 mm holes have three times the surface area in contact with marrow compared to the 2 mm deep holes. In addition, the epiphysial scar of the rabbit trochlea is ~3 mm deep such that the deep holes penetrated through it while the shallow holes did not. It has been previously suggested that successful wound healing and cartilage repair relied on increased recruitment of bone marrow-derived mesenchymal cells and that different cell types may reside in specific regions of the marrow. In this study, deep DRL created more access channels to the marrow and may potentially recruit a greater number of cells and a variety of cell types from the deep marrow stroma, resulting in improved cartilage repair (Fig. 2). We believe that the statistically significant one-third increase in %Fill detected in the deeper DRL6 versus the shallower DRL2 defects is functionally important in terms of the ability of the repair tissue to bear load, since many clinical marrow stimulation studies only show partial fill of the cartilage lesion that is most likely not effectively load-bearing. The higher %Col2 and %GAG and lower %Col1 observed in the DRL6 defects relative to DRL2 defects (Fig. 2) may also render the repair tissue more durable and able to bear load.

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DISCUSSION

Surgical technique is expected to be an important factor in determining the success of bone marrow stimulation procedures. It is now well established that the calcified cartilage layer should be removed to provide access to bone marrow which is the principle source of repair cells. To our knowledge, the present study is the first to investigate the effect of subchondral perforation depth on cartilage repair outcomes, and to directly compare drilling and microfracture techniques. The results confirmed our hypotheses that drilling deeper increases cartilage repair quantity and quality in a statistically significant manner; however, we did not observe significant improvement in tissue repair comparing drilling to microfracture at the same 2 mm penetration depth.

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the subchondral tidemark and bone plate, but in young rabbits at 24 weeks postoperatively. Some of their findings were in contrast to our observations of relatively poor basal and lateral integration regardless of hole type and hole depth, possibly due to the ability of the younger skeletally immature animals used previously to repair robustly. Poor cartilage repair outcomes after DRL observed previously in clinical studies may have been due to incomplete debridement of the calcified layer or excessive removal of subchondral bone. Some of the first clinical drilling procedures with poor outcome also involved large arthrotomies, concomitant synovectomies, meniscectomies, osteophyte, and cartilage shaving, along with loss of a large volume of subchondral bone, pointing out the importance of designing specific and well-controlled clinical studies of cartilage repair. Poor repair reported from some animal studies may have also been due to aggressive subchondral invasion or ablation of the subchondral bone plate (2 mm deep debridement), leading to bone resorption, cyst formation and even collapse. These findings highlight the importance of preserving the integrity of a healthy subchondral bone plate and underlying trabecular structure in marrow stimulation procedures.

Concerning animal models for cartilage repair studies, the use of skeletally immature rabbits is not recommended since they display a very high propensity for spontaneous repair unlike that seen in adult human cartilage repair. Our skeletally mature rabbit model was carefully chosen and the study design was based on simulating clinical microfracture in a geometrically proportional manner to human. For example, our surgical tool design, defect and hole pattern as well as their placement resulted in 17% of the defect surface area being perforated over the entire defect area, which is within the hole perforation density range performed clinically (13–49%, estimated from literature). We were also aware through our unpublished findings of some potentially important species differences which provide some justification for the use of a mature rabbit model in cartilage repair. In bone marrow stimulation-based repair, the state of the subchondral bone, being the primary source of repair, may be more important than the properties of remaining cartilage. We compared subchondral bone structure in human condyles to adult rabbit trochlea, and found a surprising similarity in subchondral porosity, pore surface area density and thickness of the bone plate between two species. On the other hand, the subchondral bone in human chronic cartilage lesions and in OA can become denser and more sclerotic and contain stem cell pools with less chondrogenic potential, which are different from our fresh defect model. To better relate animal models to human repair, alternative injury-induced chronic cartilage defects could be used in future studies.

Our study revealed that the specific surgical technique used can influence cartilage repair outcomes in an important manner. Drilling deeper to 6 mm versus 2 mm improved repair tissue quantity and quality in a statistically significant fashion, while drilling compared to microfracture at the same 2 mm depth produced similar repair outcomes. Our results support the notion that marrow stimulation procedures could be further optimized in a clinical setting to improve cartilage repair outcomes.

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