ABSTRACT: Bone marrow stimulation is performed using several surgical techniques that have not been systematically compared or optimized for a desired cartilage repair outcome. In this study, we investigated acute osteochondral characteristics following microfracture and comparing to drilling in a mature rabbit model of cartilage repair. Microfracture holes were made to a depth of 2 mm and drill holes to either 2 mm or 6 mm under cooled irrigation. Animals were sacrificed 1 day postoperatively and subchondral bone assessed by histology and micro-CT. We confirmed one hypothesis that microfracture produces fractured and compacted bone around holes, essentially sealing them off from viable bone marrow and potentially impeding repair. In contrast, drilling cleanly removed bone from the holes to provide access channels to marrow stroma. Our second hypothesis that drilling would cause greater osteocyte death than microfracture due to heat necrosis was not substantiated, because more empty osteocyte lacunae were associated with microfracture than drilling, probably due to shearing and crushing of adjacent bone. Drilling deeper to 6 mm versus 2 mm penetrated the epiphyseal scar in this model and led to greater subchondral hematoma. Our study revealed distinct differences between microfracture and drilling for acute subchondral bone structure and osteocyte necrosis. Additional ongoing studies suggest these differences significantly affect long-term cartilage repair outcome.

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

A recent retrospective study on 25,124 knee arthroscopies demonstrated evidence of chondral defects in 15,074 knees (60%), and up to 67% were classified as localized and focal defects.1 These cartilage defects have poor intrinsic healing capacity and, if left untreated, can lead to joint degeneration, chronic pain, and disability. Several surgical repair modalities have been developed to treat focal cartilage defects.5 The most widely practiced are bone marrow stimulation techniques such as subchondral drilling,3 abrasion arthroplasty,4 and microfracture (MF).5 The rationale behind these methods is to initiate bleeding and wound repair via small fractures in subchondral bone. Drilling, first introduced by Pridie in 1959,3 was prevalent for decades4,6 prior to needles, which were then introduced to penetrate the subchondral plate, and is thought to be superior to Pridie drilling,5,10,11,16 which employs a hand-driven or motorized drill that may produce heat necrosis in the bone—a hypothesis that has never been tested. The MF technique represents a first-line treatment option for full-thickness cartilage defects in the knee,12,17 showing a decrease in pain and improved knee function after 1- or 2-year follow-up.7–11,18 Recent clinical data has also provided evidence that outcomes after MF are similar to those of autologous chondrocyte implantation.17–19

In marrow-stimulating procedures, perforations to the subchondral bone allow the influx of blood and marrow-derived cells into the holes and defect followed by the formation of a blood clot. The subsequent classical wound repair cascade comprised of an acute inflammatory response and cell chemotaxis leads to the generation of a vascularized granulation tissue, and the proliferation of pluripotent mesenchymal progenitor cells with a capacity to differentiate into multiple mesenchymal cell types.20 Bone remodeling can proceed along with the inductive and reparative response and cell chemotaxis leads to the generation of a vascularized granulation tissue, and the proliferation of pluripotent mesenchymal progenitor cells with a capacity to differentiate into multiple mesenchymal cell types.20 Bone remodeling can proceed along with the inductive and reparative response and cell chemotaxis leads to the generation of a vascularized granulation tissue, and the proliferation of pluripotent mesenchymal progenitor cells with a capacity to differentiate into multiple mesenchymal cell types.20 Bone remodeling can proceed along with the inductive and reparative response and cell chemotaxis leads to the generation of a vascularized granulation tissue, and the proliferation of pluripotent mesenchymal progenitor cells with a capacity to differentiate into multiple mesenchymal cell types.20 Bone remodeling can proceed along with the inductive and reparative response and cell chemotaxis leads to the generation of a vascularized granulation tissue, and the proliferation of pluripotent mesenchymal progenitor cells with a capacity to differentiate into multiple mesenchymal cell types.

The aim of the current study was to compare acute osteochondral characteristics 24 h after microfracture (MF) or microdrilling (MD) procedures in a skeletally mature rabbit model. We tested the hypotheses that 1) microfracture induces bone compaction around holes, and 2) microdrilling induces heat necrosis around holes. The effect of hole depth was also examined where MF was performed to a depth of 2 mm (MF2), while MD was performed to a depth of either 2 mm (MD2) or 6 mm (MD6). Our results revealed significant differences between MF and MD, which could potentially influence subsequent repair responses and longer term cartilage repair properties.
MATERIALS AND METHODS
Skeletally mature (10-month-old) female New Zealand White rabbits (Charles River, St. Constant, Canada) were used for this study. The research protocol was reviewed and approved by the Animal Care Committee of the University of Montreal. Following anesthesia with intramuscular injection of ketamine/xylazine/buprenorphine and inhalation of 3% isoflurane/oxygen, each animal underwent sequential bilateral arthroscopies with a medial parapatellar incision, and a cartilage defect of 4 mm × 4 mm was created in the center of the trochlear groove. The defect was completely debrided of the calcified cartilage using flat surgical blades (Fine Science Tools Inc., North Vancouver, Canada). MD and MF techniques were applied on each chondral defect using customized surgical tools (Fig. 1A,B) to create four perforating holes, each spaced by 0.5–1.0 mm, into subchondral bone. The mini-microfracture awl custom-machined from titanium rods (TI017905, Goodfellow Corporation, Oakdale, PA) bore a stopper at 2 mm from the tip, and pierced holes with a conical shape 2-mm deep with a 1-mm base diameter (referred to as MF2 holes; Fig. 1C). The commercial drill bits of 0.9-mm diameter (19008-14, Fine Science Tools Inc., North Vancouver, Canada) were custom-machined to bear a stopper that permitted depth control of the MD hole to either 2 mm or 6 mm (referred to as MD2 or MD6 holes; Fig. 1D). Cylindrical holes were made by using a high-speed microdrill (18000-17, Fine Science Tools Inc., North Vancouver, Canada) according to a predesigned hole pattern (Fig. 1E). Continuous irrigation with sterile Ringer’s Lactate solution (RLS) from a squeeze bottle, precooled on an ice bath, was applied during drilling to limit heating. The hole pattern was designed in order to have a minimum of N = 4 holes of each type (MF2 = 4, MD2 = 8, MD6 = 4) with two animals, each hole being treated as a sample for analysis of acute characteristics. This design also allowed side-by-side comparison of MD2 to MF2 at distal holes and MD2 to MD6 at proximal holes (Fig. 1E). The extent of bleeding from holes was scored as 0, 1, and 2, corresponding to no bleeding, some bleeding in holes, and heavy bleeding, respectively. The defect was rinsed extensively with RLS to remove loose bone and cartilage debris. A similar hole pattern was applied to the condyles. The patella was then repositioned and the knee closed in sutured layers. Animals were allowed immediate unrestricted ambulation in cages after recovery, and received buprenorphine analgesia twice at approximately 1 h and 16 h following arthroscopy.

Rabbits were sacrificed 1 day postoperatively by an overdose of sodium pentobarbital (381, CDMV, St-Hyacinthe, Quebec, Canada). Distal femora were fixed in 80% ethanol at 4°C. Fixed defects were scanned by micro-CT (Skyscan X-ray Microtomography 1172, Kontich, Belgium) at 10 μm/pixel, followed by methylmethacrylate (MMA) embedding. MMA sections, 6 μm thick, were taken from the middle of the holes (Microtome Leica SM 2500), deplastified in ethylene glycol monoethyl-ester acetate (Fisher Scientific) and stained with Goldner’s Trichrome prior to digital imaging. Osteocyte necrosis was evaluated by counting empty osteocyte lacunae in a 0.2-mm wide region along hole borders and from regions far away (1–2 mm) from the holes as controls. To quantify bone compaction around holes, micro-CT images were reconstructed to measure bone density in sleeves 0.2-mm wide and 1-mm deep, surrounding distal MD2 and MF2 holes, using the 3D analysis function in CTAn software (Skyscan, Kontich, Belgium). Line measurements of trabecular thickness around these holes was also performed at four fixed positions (0, 3, 6, 9 o’clock) and at three different vertical levels of top, mid, and low that were each separated by 0.3 mm, with the top level being 0.5 mm from the debrided base of the defect.

Numerical values are shown as mean ± standard deviation (n = 4) comparing MD2 and MF2. Statistical analyses used the two-tailed paired Student’s t-test with p < 0.05 considered significantly different.

RESULTS
In this rabbit model, the debridement step completely removed all calcified cartilage to expose bone at the defect base such that slight punctuate bleeding was observed immediately following debridement. On average, heavy bleeding was found from MD6 holes (mean score 1.8), some bleeding from MD2 holes (mean score 1.0), and no immediately visible bleeding in three out of four MF2 holes (mean score 0.3). Histology at 1 day postoperation revealed blood clots covering the osteochondral defects and filling holes created by both MF and MD techniques (arrows in Fig. 2). Acute subchondral hematoma was confined to the void volumes created by the holes for both MD2 and MF2 defects, but occupied a greater volume for the MD6 holes which broke through the epiphysial scar (the closed growth plate) and reached the deep marrow cavity (asterisk in Fig. 2).

MF induced acute fracturing and compaction of the bone surrounding the holes, leaving dense bone at the periphery of all MF2 holes (solid arrowheads in Figs. 2, 3, and 4), whereas MD removed bone and bone debris from holes and left clean cut borders in all MD holes (empty arrowheads in Figs. 2, 3, and 4). Free access channels from MD holes to marrow space were present from top to
deep regions of the MD holes (empty arrowheads in Figs 2, 3, and 4), in contrast to the MF2 holes that were largely sealed off by fractured and compacted bone (solid arrowheads in Figs 2, 3, and 4) leaving little or no access to marrow space. Analyses from micro-CT images revealed that bone density in 0.2-mm thick and 1-mm deep sleeves surrounding the holes, was significantly higher for MF2 than MD2 ($p = 0.02$, Fig. 5A). Trabecular thickness

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**Figure 2.** Goldner’s Trichrome-stained MMA sections of osteochondral defects with marrow-stimulating 2-mm deep microdrill (MD2) holes versus 2-mm deep microfracture (MF2) holes (A, B) in the distal location, and 2-mm deep MD2 versus 6-mm deep MD6 microdrill holes (C, D) in the proximal location of the trochlea of rabbits sacrificed at 1 day postoperatively. Arrows point to the blood clots covering the defects. Solid arrowheads show the dense and crushed bone around MF2 holes, and empty arrowheads point to marrow openings in MD2 and MD6 holes. Asterisk indicates penetration of epiphyseal scars (closed growth plates) by MD6 holes. Scale bar = 1 mm.

**Figure 3.** Vertical micro-CT images of the trochlea from rabbits sacrificed at 1 day postoperatively. See the Figure 2 legend for symbol definitions. Scale bar = 1 mm.
around MF2 holes, that includes compacted bone, was also significantly higher than around MD2 holes, at 250 ± 40.1 μm versus 136 ± 45.7 μm, respectively (p = 0.03, Fig. 5B).

Histology showed that the bone matrix surrounding MD holes remained morphologically normal and intact at 1 day postoperative, in contrast to the bone matrix surrounding microfracture holes which was heavily fractured (Fig. 6). The osteocytes around MD holes appeared largely viable according to their morphology (Fig. 6A,B) with a few empty osteocyte lacunae (red arrows in Fig. 6A,B). In contrast, there were significantly more empty osteocyte lacunae in the fractured and crushed bone adjacent to the MF holes (red arrows in Fig. 6C,D). Specifically, 27.7 ± 5.25% of osteocytes found in trabecular bone adjacent to distal MF2 had empty lacuna, in contrast to 12.0 ± 4.45% found with the MD2 holes (p = 0.004). In trabecular bone in control regions 1–2 mm distant from the holes, 9.7 ± 1.04% of osteocytes had empty lacuna, a level that was not significantly different from that adjacent to drilled holes (Fig. 5C).

**DISCUSSION**

This study is the first to date to directly compare the acute effects of microfracture and drilling, two widely practiced marrow-stimulating procedures for cartilage repair that have inherent mechanical differences which may result in important clinical differences for cartilage repair outcomes. Here, we focused only on acute events at 1 day postoperative in order to accurately characterize the state of the subchondral bone just after perforation and prior to analyzing longer term consequences. Based upon literature and the mechanical action of microfracturing (MF) and microdrilling (MD), we hypothesized that MF would produce dense compacted bone around holes, more so than MD, and that MD would produce more heat necrosis than MF. The first hypothesis was substantiated by evidence of the creation of a sleeve of dense compacted bone surrounding MF holes both in histology (Fig. 2) and in micro-CT
(Figs. 3, 4, and 5A, B). The second hypothesis, however, was not supported by our data since we found that MF produced more empty osteocyte lacunae than MD (Figs. 5C and 6), apparently due to mechanical shearing and crushing of bone. Our drilling procedure which involved cooled irrigation did not produce apparent heat necrosis in adjacent bone. We also found that deeper holes, as expected, produced greater subchondral hematoma with increased access to marrow stroma (Fig. 2).

Significant thermal effects on bone during orthopedic interventions have long been recognized, where a rise in temperature to 50°C at the surgical site causes bone necrosis. Research in rat, canine, and human bone has shown that thermal damage delays bone healing, increases bone resorption, and can cause implant loosening or failure. Consequently, a variety of improved drill bits and finely tuned drilling parameters have been developed to reduce thermal necrosis, although irrigation, both cooled and room temperature, remains the simplest approach. The contour and shape of drill burs influences the extent of thermal damage where some commercial drill bits produce less heat than standard equivalents. Kirschner-wires (or K-wires) are also commonly used, but have a smooth surface without threads and therefore do not remove bone debris, but compress it, leading to increased bone density (as in the MF of our study) and increased heat due to friction. In our study, we minimized heating by using a twist drill which removed bone debris effectively, along with continuous irrigation with a cooled buffer. These aspects help preserve the vitality of surrounding bone after MD, and with minimal necrosis (Fig. 6A, B). Conversely, Figure 6C, D demonstrates that roughly twice as many osteocytes near MF holes exhibited characteristics of necrosis than around MD holes (Fig. 5C). Osteocyte necrosis is commonly observed in bone fracture, where these cells display cytoplasmic swelling, rupture or loss of plasma membrane, and enlargement of lacunae or organelle dissolution. It is plausible that the disturbance of the bone matrix during MF and the disconnection of osteocytes from neighboring cells as well as direct mechanical damage and shear to osteocytes induced cell death around MF holes. These results suggested that crushing and fracturing of bone by microfracture induced substantial osteocyte necrosis, whereas drilling did not produce apparent heat necrosis when holes were made using a properly designed drill bit under cooled irrigation. To our knowledge, this finding has not been previously reported.

Bone marrow stimulation procedures for cartilage repair should promote blood flow and blood clot formation in the debrided cartilage lesion and allow for an influx of cells from the bone marrow. Although bleeding is often seen clinically in MF when the pressure of circulating arthroscopic fluid is reduced, we found overt bleeding at the time of surgery in only one out of four MF holes in our study. Nonetheless, 24 h later, all holes will fill with a blood clot suggesting continual bleeding or perhaps additional fracturing from weight bearing postsurgically. We found that MF compacted surrounding bone and largely sealed off MF holes (Figs. 2, 3, and 4). This sealing off may have limited both bleeding and subsequent access of marrow cells to the MF defect, potentially impeding subsequent repair processes, in contrast to MD holes which appeared to openly communicate with marrow spaces. Ongoing studies suggest that the sealing off of MF holes by a layer of compact and fractured bone can reduce the quantity and quality of repair cartilage produced.
Our choice of animal model and the dimensions of the surgical tools were based on simulating human clinical practice. For example, placement of four 1-mm diameter holes in a $4 \times 4$ mm ($16 \ mm^2$) rabbit defect results in $\sim 20\%$ of the defect area being covered by holes, similar to that performed clinically where $20\% - 40\%$ can be estimated from literature.\(^{40}\) In terms of dimensions, our $16 \ mm^2$ defect is perhaps $10 \times$ smaller than a typical $1.5 \ cm^2$ human lesion treated by MF, thus justifying a scaling factor of 3 for linear dimensions, which is the ratio of diameters of the clinical tool (3 mm) and our rabbit tool (1 mm). Although rabbit model has been occasionally criticized as a cartilage repair model, these criticisms are often due to the use of skeletally immature animals which heal much more easily than mature animals that are quite useful in cartilage repair studies.\(^{23,24}\) We have also recently compared subchondral bone structure in rabbit to human where a surprisingly similar thickness of the bone plate at $\sim 200-300 \ \mu m$ and porosity at $\sim 60\%$ were observed in both species. Although the dimensions and choice of species in our study are well justified, our customized MF awl has a ledge to control hole depth (Fig. 1C); this ledge may actually sequester fractured bone to remain in the hole, possibly promoting sealing it off rather than creating a ridge around the hole. These considerations lead us to prudently interpret our results and further examine our hypotheses using additional tool designs and in larger species including human. Specifically, animal studies using custom awls with and without a ledge are being planned to further investigate the influence of specific microfracture tool designs on resulting subchondral bone structure.

In summary, we demonstrated distinct differences in acute fractures of subchondral bone between microfracture and drilling procedures. Microfracture with an awl induced fracturing and bone compaction around holes that were largely sealed off from adjacent bone marrow, in contrast to drilling which cleanly removed bone debris and left channels that communicate between the hole and marrow. Microfracture also produced a high level of osteocyte necrosis in adjacent bone, in contrast to our drilling method which included cooled irrigation and did not cause apparent thermal necrosis. The consequences of these acute differences are currently under investigation in ongoing longer term studies that could identify methods to optimize bone marrow stimulation for the repair of articular cartilage.\(^{41}\)

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REFERENCES


