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Bone marrow stimulation induces greater chondrogenesis in trochlear vs condylar cartilage defects in skeletally mature rabbits

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SUMMARY

Objective: The aim of this study was to compare the early repair response of cartilage defects in trochlea (TR) and medial femoral condyle (MFC) at 2–3 weeks after bone marrow stimulation.
Design: Bilateral full-thickness cartilage defects were generated in central trochlear groove and MFC of skeletally mature rabbits. Four subchondral perforations were made on each defect, either by microfracture to 2 mm deep, or by drilling to 2 mm or 6 mm deep. Rabbits were sacrificed either on Day 14 post-operatively or on Day 21. Defects were analyzed by histology, stereology, histomorphometry and micro-computed tomography (CT). Intact femurs ($N = 4$) served as controls.
Results: Stromal cell density recruitment was similar in all defects, irrespective of defect location and surgical techniques used. There was a robust appearance of chondrocytes at Day 21 in TR defects with significantly higher volume fraction of chondrocytes in TR compared to MFC ($P = 0.013$). Chondrogenic foci were observed in marrow penetrating holes, with a significantly higher frequency and larger foci in TR vs MFC defects at Day 21 ($P = 0.043$ and $P = 0.0014$, respectively). Micro-CT analysis showed that deep drilling elicited significantly more mineralized bone fill compared to shallower perforations at 2 and 3 weeks repair (all at $P \leq 0.0008$).
Conclusions: Bone marrow stimulation induced greater chondrogenesis in TR vs MFC defects in adult rabbits, with more chondrocytes and larger chondrogenic foci appearing in TR vs MFC on Day 21 post-operation.

Introduction

Articular cartilage lesions are a common pathology found in knee arthroscopy[1] that can cause serious limitations in daily function and may lead to symptomatic joint degeneration[2]. In human, the weight-bearing medial femoral condyle (MFC) is the most commonly affected area, and is the predominant location for severe full-thickness lesions[1,3]. Trochlear (TR) lesions are less frequently detected but are more challenging to treat because of other associated pathologies of the patellofemoral joint[4]. While an optimal treatment algorithm remains elusive[5], it is generally accepted that the treatment choice for repair is guided by patient- and defect-specific factors, and that the anatomic location of the defect is an important variable influencing clinical outcome[6]. Nonetheless, the factors that influence repair outcome at different locations are still largely unknown.

Over the past decades, many surgical approaches have been developed that aim to restore the hyaline articular cartilage surface and to permit subchondral bone regeneration. Bone marrow stimulation techniques such as microfracture[7] and subchondral drilling[8] involve the surgical placement of holes that connect the debrided cartilage lesion to the underlying bone marrow stroma to recruit pluripotential cells capable of repairing soft and hard tissues. The mechanisms and early healing response of bone marrow stimulation have been studied in animal models by our group and by others[9–16]. Increased recruitment of marrow-derived stromal cells was linked to improved cartilage repair in rabbit models[10]. Chondrogenic foci arising from subchondral bone progenitors has also been identified as the main source of cartilaginous repair tissue

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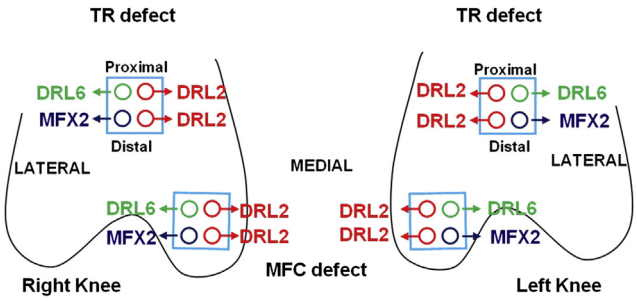
for the chondral defects in bone marrow stimulation animal models and is therefore the central mediating feature for successful cartilage repair[9]. Osteoclasts may play a key role in marrow stimulated cartilage repair given their unique ability to resorb bone by forming a ruffled seal at the bone surface and releasing tartrate-resistant acid phosphatase (TRAP)[17]. Previous studies found that TRAP is associated with osteoblast migration to bone resorption sites, and may initiate osteoblast differentiation, activation and proliferation[18,19]. Also, in a cartilage repair rabbit model, osteoclasts were elicited following subchondral perforation and were involved in resorption of damaged bone, remodeling of new woven bone and cell recruitment, which promoted bone marrow-derived cartilage repair integration[16].

We have previously reported that the specific surgical technique used for marrow stimulation can also lead to different bone structure and influence repair outcomes[20–22]. Microfracture (MFX) induced bone compaction, fracturing and cell necrosis around holes whereas a deeper drilling approach enhanced marrow access and produced improved repair compared to shallower perforation in rabbit TR, but not in rabbit condyle[23] (unpublished data). When deeply drilled, TR defects communicate with the metaphyseal bone[24]. We have also analyzed the structure of TR and MFC from intact femurs of skeletally mature rabbits and have found thicker cartilage and subchondral bone plate, and higher subchondral bone density in MFC than in TR[25] (unpublished data), some of which may be factors predisposing for poor vs successful repair in this acute defect cartilage repair model. Based on these observations, this study tested the hypotheses that (1) bone marrow stimulation recruits more marrow-derived stromal cells in TR vs MFC defects, (2) bone marrow stimulation induces greater chondrogenesis in TR vs MFC defects, and (3) drilling induces more chondrogenesis compared to MFX.

Materials and methods

Experimental design and rabbit surgical model of bone marrow stimulation

The research protocol was reviewed and approved by an institutional ethics committee for animal research. A bilateral model was chosen to minimize the influence of inter-animal variation and to reduce the number of animals. Six skeletally mature (10-month-old) female New Zealand White rabbits (Charles River, St. Constant, Canada) underwent bilateral arthrotomies with a medial parapatellar incision after anesthesia. Cartilage defects were created in the central trochlear groove (TR) and in the MFC of each animal by manual curettage, with complete debridement of the calcified cartilage to expose subchondral bone with visible punctate bleeding. By using customized surgical tools such as drill burrs (0.9 mm diameter) and an awl (1 mm base diameter) described previously[20], four subchondral perforations were made on each defect in TR and in MFC: one 6 mm deep drill hole (DRL6) and one 2 mm deep drill hole (DRL2) in the proximal zone of the defect, and one microfracture hole (MFX2) and one drill hole (DRL2), both to 2 mm deep, in the distal zone, which provides a total of 48 marrow stimulating holes with a minimum of 12 holes of each type in TR and in MFC (Fig. 1). Constant irrigation with cooled sterile Ringer's lactate solution was applied to minimize heating and prevent heat necrosis during drilling[20], and to remove loose bone debris before knees were closed in sutured layers. Animals were housed individually in cages with a dimension of 0.6 m (L) × 0.6 m (W) × 0.4 m (H), and received buprenorphine analgesia twice at approximately 1 h and 16 h following arthrotomy. No peri-operative antibiotics were given, and no immobilization/cast was applied on the animals post-operation. All animals were closely monitored for infections and other



Sacrifice Time Point	Number of Animals	Number of Surgical Holes			Total Number of Holes
		DRL6	DRL2	MFX2	
Day 14	3	6	12	6	24
Day 21	3	6	12	6	24
Total	6	12	24	12	48

Fig. 1. Experimental design and placement of surgical holes. Cartilage defects were created bilaterally in both central trochlear groove (TR) and MFC in six rabbits. Each defect received four subchondral perforations, one 6 mm deep drill hole (DRL6) and one 2 mm deep drill hole (DRL2) in the proximal part of the defect, and one microfracture (MFX2) and one drill holes (DRL2), both to 2 mm deep, in the distal part, providing a total of 48 marrow stimulating holes with a minimum of 12 holes of each type in TR and in MFC. Six knees were collected at Day 14 from three rabbits and six knees at Day 21 post-operatively.

complications. Two out of six rabbits appeared slightly quiet in the first few days after surgery, while others remained active, able to hop inside the cages. Starting from Day 7 post-surgery, effusions or bumps were observed in all operated knees, mainly at the incision site, and gradually lessened after 14 days, though bumps were still seen in 33% of knees (two out of six) on Day 21. No signs of infection in any knee were observed. Operated animals were randomly assigned to two sacrifice time points. Six knees were collected on Day 14 from three rabbits, and six knees on Day 21 post-operatively (Fig. 1). A DRL2 hole (from an MFC defect and for Day 21 repair) was lost during histological sectioning and excluded from analyses except the micro-computed tomography (CT) analysis. Another two skeletally mature rabbits (four knees) received no surgical intervention and served as intact controls (Day 0).

Micro-CT scanning, histoprocessing, histostaining, immunohistochemistry and enzymatic staining for TRAP+ osteoclasts

Collected femoral ends were fixed in 80% ethanol at 4°C, micro-CT scanned (Skyscan X-ray Microtomography 1172, Kontich, Belgium) with an isotropic voxel size of 10 μm, and then histo-processed for non-decalcified methylmethacrylate (MMA) embedding. Transverse sections (6 μm thick) were collected from the central areas of the proximal and distal sets of holes in each defect (Microtome LeicaSM2500). MMA sections were also collected from similar regions in intact TR and MFC. Histostaining with Goldner's Trichrome and Safranin-O/Fast Green/iron hematoxylin, immunostaining for collagen type II and enzymatic staining for TRAP were performed as previously reported[16].

Stereological analysis of volume density of stromal cells and chondrocytes in holes

Cell volume densities (Vv) of bone marrow-derived stromal cells and chondrocytes were quantified in repair tissue formed in the