Small Subchondral Drill Holes Improve Marrow Stimulation of Articular Cartilage Defects

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Introduction: Marrow stimulation techniques such as subchondral drilling are first-line treatment options for symptomatic small articular cartilage defects [1]. Their guiding principle is to establish a communication with the subchondral bone marrow compartment, allowing for the immigration of pluripotent progenitor cells during articular cartilage repair [2]. Despite the long-time clinical use of subchondral drilling [3], no specific recommendations on hole size exist. Smaller instruments are more commonly used [4] and may induce less disturbance of the subchondral bone microarchitecture, but larger holes allow for an amplified access of reparative elements to the cartilage defect [5]. Here, we tested the hypothesis that osteochondral repair is improved when the subchondral bone is perforated by small drill holes that reflect the physiological subchondral trabecular distance. Furthermore, we hypothesized that the extent of articular cartilage repair correlates with subchondral bone reconstitution.

Methods: All experiments on the skeletally mature, healthy, female Merino sheep (n = 20; mean age 36 ± 12 months; average body weight 72.2 ± 17.3 kg) were approved by the local governmental animal care committee.

First, the physiological trabecular distance in the normal ovine subarticular spongiosa (n = 6 sheep) was determined by micro-CT (Skyscan 1172; Skyscan, Kontich, Belgium; tube voltage 70 kV, current 139 µA, 0.5 mm aluminium/copper filter; 0.4° intervals; 2,400 ms exposure time; spatial resolution 15 µm).

Next, rectangular full thickness chondral defects (4 x 8 mm) were created unilaterally on the lateral facet of the femoral trochlea in 14 animals and outlined down to the cement line. Standardized subchondral drilling (n = 6 drill holes per defect) was performed using K-wires under constant irrigation applying two different drill hole diameters: a small diameter reflective of the physiological trabecular distance (1.0 mm, referred to as 1.0 mm group; n = 7), and a large diameter representing a non-physiological trabecular distance (1.8 mm group; n = 7). Postoperatively, animals were allowed immediate full weightbearing. One animal was excluded due to deep wound infection (1.8 mm group).

After 6 months in vivo, articular cartilage repair was evaluated by 2-4 blinded investigators macroscopically (0-20 points) and histologically on 130 safranin orange/fast green (safranin O) and hematoxylin/eosin (HE) stained sections (0-31 points) using established scoring systems [6, 7]. Osteoarthritic changes of the adjacent cartilage were scored (0-25 points) on 39 histological sections [8]. Type-I and type-II collagen contents were evaluated semiquantitatively (0-4 points) by immunohistochemistry applying monoclonal mouse anti-type-I or anti-type-II collagen IgG (both Acris, Hiddenhausen, Germany) and a biotinylated secondary anti-mouse antibody (Vector Laboratories, Burlingame, CA). Alterations of the subchondral bone plate and the subarticular spongiosa were
separately assessed by micro-CT following standardized segmentation into volumes of interest as previously described [9]. Intralesional osteophytes and subchondral bone cysts were quantified. The two-sample t-test served to compare between the 1.0 mm and 1.8 mm group. The strength of association between key micro-CT parameters of the subchondral bone and the histological cartilage grading was determined using Spearman’s correlation coefficient (r). A two-tailed \( P < 0.05 \) was considered significant (OriginPro 8G, OriginLab Corporation, Northampton, MA).

**Results:** The physiological trabecular separation in the normal subarticular spongiosa of the ovine trochlea was of 0.9 ± 0.1 mm.

Macroscopic evaluation of the cartilaginous repair tissue revealed no significant differences between 1.0 mm and 1.8 mm drill holes for all individual parameters and the inverse average total score (1.0 mm group: 5.1 ± 3.1; 1.8 mm group: 6.7 ± 3.1 points; \( P = 0.24 \); Figure 1).

Application of 1.0 mm subchondral drill holes led to significantly improved histological matrix staining (\( P = 0.04 \)), cellular morphology (\( P = 0.02 \)), subchondral bone reconstitution (\( P = 0.04 \)) and inverse average total histological score (1.0 mm group: 19.2 ± 4.8; 1.8 mm group: 25.4 ± 3.7 points, \( P = 0.02 \); Figure 1).

Analysis of osteoarthritic changes in the cartilage adjacent to the defects revealed no significant differences (1.0 mm group: 15.5 ± 4.3; 1.8 mm group: 12.6 ± 4.2; \( P = 0.32 \)).

Immunoreactivity to type-II collagen was significantly stronger in 1.0 mm drill holes (1.9 ± 1.4) compared to 1.8 mm drill holes (0.3 ± 0.5; \( P = 0.04 \)) while immunoreactivity to type-I collagen was significantly reduced (1.0 mm group: 2.9 ± 0.9; 1.8 mm group: 4.0 ± 0.0; \( P = 0.01 \); Figure 2).

Restoration of the microstructure of the perforated subchondral bone plate was significantly improved after 1.0 mm compared to 1.8 mm drilling (Figure 1), as shown by higher bone volume (BV/TV; 44.2 ± 9.5 versus 29.5 ± 11.0%; \( P = 0.03 \)) and reduced subchondral bone plate thickening (Ct.Th; 0.2 ± 0.1 versus 0.4 ± 0.1 mm; \( P = 0.01 \)). Likewise, the microarchitecture of the subarticular spongiosa was better restored following 1.0 mm drilling, indicated by significantly higher BV/TV (38.2 ± 5.6 versus 28.9 ± 7.2%; \( P = 0.03 \)), and more and thinner trabeculae (Th.N: 2.6 ± 0.5 versus 1.7 ± 0.3; Tb.Th: 0.4 ± 0.1 versus 0.6 ± 0.1 mm; both \( P < 0.01 \)). Moreover, the bone mineral density (BMD) of the subchondral bone in the 1.0 mm group was similar to the adjacent subchondral bone (\( P > 0.06 \)), whereas it was significantly reduced for 1.8 mm drill holes (\( P < 0.03 \)). Intralesional osteophytes were detected in 23% of all defects, homogenously distributed between groups. No subchondral bone cysts were found.

No significant correlations existed between micro-CT parameters of subchondral bone and histological parameters of articular cartilage repair (all \( P \geq 0.11 \)).

**Discussion:** Small subchondral drill holes that reflect the physiological trabecular distance improve osteochondral repair more effectively than larger drill holes in a translational animal model at 6 months postoperatively. No significant correlations existed between articular cartilage and subchondral bone repair.

**Significance:** Taking into consideration that the physiological trabecular separation of human (0.8 ± 0.2 mm) [10, 11] and sheep subarticular spongiosa is similar, the translational data presented here have important and direct clinical implications for the use of marrow stimulation techniques in patients and warrant prolonged follow-up investigations.
Figure 1. Macroscopic, histological, and micro-CT analysis of the osteochondral unit. Macroscopic evaluation revealed no significant differences between 1.0 mm and 1.8 mm drill holes (A-C). The histological analysis of safranin O (D-I) and HE (J-L) stained sections indicated significantly improved repair for 1.0 mm drill holes. Micro-CT assessment showed insufficiently restored subchondral bone for 1.8 mm drill holes (M-O). Images represent the mean value of the respective treatment group. Scale bars: 0.5 mm (G-L), 1.0 mm (D-F), 2.0 mm (A-C), and 3.0 mm (M-O). Arrowheads indicate defect borders.
**Figure 2.** Immunohistochemical evaluation of the articular cartilage. Immunoreactivity to type-II collagen (A-F) was significantly stronger, while immunoreactivity to type-I collagen (G-L) was significantly weaker for 1.0 mm drill holes compared with 1.8 mm drill holes. Images represent the mean value of the respective treatment group. Scale bars: 1.0 mm (A-C, G-I), 0.5 mm (D-F, J-L). Arrowheads indicate defect borders.