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Biomechanical Properties of Repair Cartilage Tissue Are Superior Following Microdrilling Compared to Microfracturing in Critical Size Cartilage Defects

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Abstract. *Background/Aim: Common surgical treatment options for large focal chondral defects (FCDs) in the knee include microfracturing (MFX) and microdrilling (DRL). Despite numerous studies addressing MFX and DRL of FDCs, no in vivo study has focused on biomechanical analysis of repair cartilage tissue in critical size FCDs with different amounts of holes and penetration depths. Materials and methods: Two round FCDs (d=6 mm) were created on the medial femoral condyle in 33 adult merino sheep. All 66 defects were randomly assigned to 1 control or 4 different study groups: 1) MFX1, 3 holes, 2 mm depth; 2) MFX2, 3 holes, 4 mm depth; 3) DRL1, 3 holes, 4 mm depth; and 4) DRL2, 6 holes, 4 mm depth. Animals were followed up for 1 year. Following euthanasia, quantitative optical analysis of defect filling was performed. Biomechanical properties were analysed with microindentation and calculation of the elastic modulus. Results: Quantitative assessment of defect filling showed significantly better results in all treatment groups compared to untreated FCDs in the control group ($p<0.001$), with the best results for DRL2 (84.2% filling). The elastic modulus of repair cartilage tissue in the DRL1 and DRL2 groups was comparable to the adjacent native hyaline cartilage, while significantly inferior results were identified in both MFX groups (MFX1: $p=0.002$; MFX2: $p<0.001$). Conclusion: More defect filling and better biomechanical properties of the repair cartilage tissue were identified for DRL compared to MFX, with the best results for 6 holes and 4 mm of penetration depth. These findings are in contrast to the current clinical practice with MFX as the gold standard and suggest a clinical return to DRL.*

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Focal chondral defects (FCDs) are very common in the knee joint, and surgical therapy for larger defects is crucial to avoid the development of early osteoarthritis. Multiple surgical techniques exist, including microfracture (MFX) and microdrilling (DRL) (1). Despite the availability of newer techniques, such as autologous chondrocyte transplantation (ACT) or autologous matrix-induced chondrogenesis (AMIC), MFX and DRL are still considered viable first-line therapy options, especially due to their excellent availability, easy surgical technique, and low cost (2-5).

In recent decades, MFX has been preferred over DRL because of good short-term results and concerns about potential thermal necrosis during drilling. However, despite good initial results, less favourable outcomes were observed in the long run (6-8). Thus, more recently, a return to DRL has been observed based on the hypothesis that DRL facilitates a deeper penetration and stimulation of bone marrow cells with higher regenerative potential. In a recent systematic review of basic science studies, Kraeutler and colleagues confirmed this hypothesis and reported a higher volume of repair cartilage tissue with slightly improved histological characteristics after DRL of FCDs (1).

Most studies, however, focused on histopathological evaluation despite the obvious importance of proper

Table I. *Overview of study groups: two FCDs were created in one knee of each animal and randomly assigned to four treatment groups (MFX1, MFX2, DRL1, DRL2) and one control group.*

	Number of FCDs (n)	Surgical Technique (MFX *vs.* DRL)	Number of holes	Penetration depth (mm)	Percentage of area holes of FCD size (%)
Control	12	-	-	-	-
MFX1	12	MFX	3	2	19
MFX2	14	MFX	3	4	19
DRL1	16	DRL	3	4	19
DRL2	12	DRL	6	4	38

MFX: Microfracturing; DRL: microdrilling; FCD: focal chondral defect.

biomechanical properties of repair cartilage tissue, especially considering long-term results. Furthermore, the number of holes for either technique, MFX or DRL, is still unclear. Steadman *et al*. recommend 9-16 holes per cm^2, while other authors suggest up to 25 holes per cm^2 (9-12). Considering the diameter of the awl or drill, the perforations comprise approximately 15-20% of the total FCD size in most recently published studies (9-11). Only Eldracher and colleagues reported holes comprising almost 50% of the FCD size (10). Uncertainty also exists for the penetration depth, with some evidence for favourable results following deeper MFX or drilling (11).

Despite numerous studies addressing MFX and drilling of FDCs, no *in vivo* study has focused on the biomechanical analysis of repair cartilage tissue following MFX and drilling in critical size FCDs with different amounts of holes and penetration depths. However, these data are critical for an optimized surgical therapy regimen. Consequently, this knowledge may unveil if the biomechanical properties of repair cartilage tissue are superior in MFX or drilling (1) and if the number of holes and the penetration depth play a significant role (2).

Materials and Methods

Animals. Thirty-three healthy and mature merino sheep (6-7 years old) were used for this study. All animals underwent the creation of two round FCDs with a diameter of 6 mm on the medial femoral condyle in the zone of the main mechanical loading. Right and left knees were randomly assigned. All 66 defects were also randomly assigned to 1 control and 4 different study groups, as shown in Table I. Animals were followed up for 1 year. The animals received water and a standard diet and were under specialized veterinary surveillance at all times. Osteoarthritis was radiologically excluded prior to inclusion. Animal experiments were conducted in accordance with the local and national legislation on the protection of animals and the NIH Guidelines for the Care and Use of Laboratory Animals. They were approved by the local governmental animal care committee and the institutional review board (AZ 55.2-1-54-2531-167-10).

Anaesthesia. Animals were sedated intravenously with diazepam (Ratiopharm, Ulm, Germany) at 0.5-1 mg/kg body weight (BW) and anaesthesia was induced by intravenous administration of 3-4 mg/kg BW propofol (Fresenius Kabi, Bad Homburg, Germany). Following endotracheal intubation, anaesthesia was maintained by inhalation of approximately 1.5% isoflurane (Abbott, Wiesbaden, Germany) and ketamine (8 mg/kg BW/h; Vetoquinol, Ismaning, Germany). Additionally, a gastric tube was inserted for the duration of the surgery. Animals received antibiotics (Cefuroxim 750mg; Hikma Pharma, Planegg, Germany) and infusions as well as sodium bicarbonate to balance the acidosis under blood gas control. Before skin suture, Metamizole (Ratiopharm, Ulm, Germany) was administered at 25 mg/kg BW to improve postoperative analgesia.

Surgical technique. The animals were placed on the operating table in the supine position with the forelimbs secured. Then, a medial parapatellar approach was used. Following an inspection of the joint to exclude the pre-existence of FCDs, 2 circular full thickness cartilage defects with a diameter of 6 mm were created on the medial condyle in the zone of the main mechanical loading with a punch and a curette. Special care was taken to avoid damage to the subchondral bone. The defects were then addressed with the different treatment options according to the randomization protocol. Study groups are summarized in Table I. To provide maximum standardization in terms of hole placement and penetration depth, custom-made surgical instruments were used (Figure 1). After thorough rinsing, the wound was closed in layers using resorbable suture material (Vicryl 2.0, Ethicon, Johnson & Johnson, Neuss, Germany). Skin closure was performed with resorbable suture material (Monocryl 2.0, Ethicon, Johnson & Johnson) as a single button suture.

Ketoprofene (Ceva, Duesseldorf, Germany; 3 mg/kg BW) was administered intramuscularly before the emergence of anaesthesia and was given for 3 days postoperatively. The animals were allowed immediate full weight bearing and were followed up for 1 year. Prior to euthanasia, a second surgery with resection of the FCDs from the medial condyle was performed. Immediately after explantation, 2 high-resolution photographs were taken from each defect. Then, all specimens were separately stored at –20˚C until further use. Anaesthesia was conducted as previously described and euthanasia was performed with intravenous administration of Pentobarbital 100 mg/kg BW and potassium chloride.

Optical measurement of defect filling. The quantitative analysis of the defect filling was performed optically using high-resolution photographs of the FCDs and the ImageJ software (Version 1.52s) (13). In brief, the initial defect size was determined, and an optical

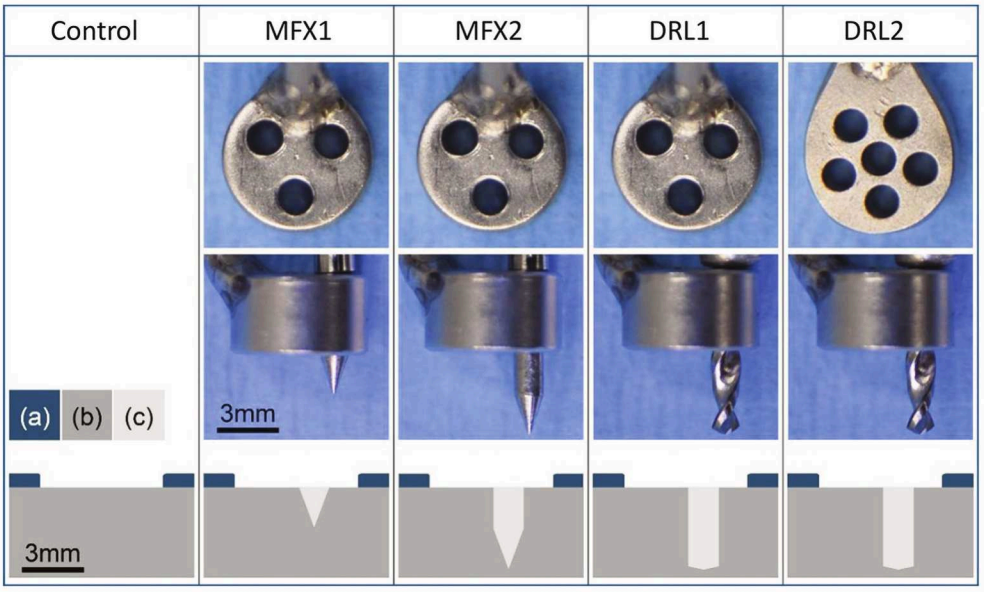

Figure 1. *Custom-made surgical instruments for standardization of hole placement and penetration depth: native cartilage (a), subchondral bone (b), and penetration of subchondral bone (c). MFX: Microfracturing; DRL: microdrilling.*

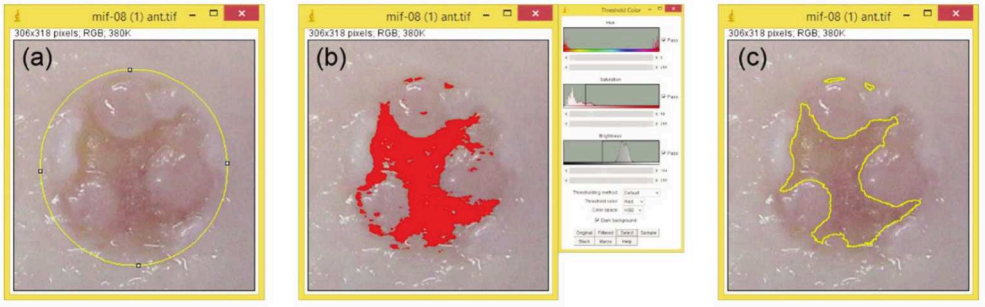

Figure 2. *Optical quantitative analysis of defect filling using ImageJ open-source software: (a) manual determination of initial defect size, (b) definition of threshold value for automated differentiation between bone and repair cartilage tissue, (c) minimal manual adjustment if necessary.*

threshold value to differentiate between bone and repair cartilage tissue was defined. Then, the percentage of FCD filling with repair cartilage tissue could be computed (Figure 2). To increase the validity of the analysis, two photos of each FCD were analysed by three independent investigators, and mean values were calculated.

Biomechanical analysis. All biomechanical analyses were performed with a custom-built dynamic indentor test system with a cylindrical indentor (d=0.8 mm) and a measurement threshold of less than 5 mN, as previously published (14). Initially, specimens were thawed in phosphate buffered saline (PBS) at room temperature for 30 min and subsequently fixed in a test chamber on a freely tiltable table, which, in combination with a high resolution 2-camera video system, facilitated a perfect horizontal alignment of the region of interest within the defect.

The thickness of the native and the repair cartilage tissue was then measured by a newly developed technique. In brief, the indentor was placed on a region without repair cartilage tissue and loaded with 5 N to ensure contact with the subchondral bone. Then, the indentor was positioned on a region with good repair cartilage tissue or native cartilage and loaded with 0.005 N to avoid compression. The thickness of the tissue was then calculated as the difference between the two indentor positions (Figure 3).

The biomechanical properties of the native and repaired cartilage tissue were then analysed by stepwise indentation with a maximum expansion of 40% (15 steps: 10 steps with 2% expansion, 5 steps with 4% expansion) at 1 mm/min and a relaxation time of 100 s (15). The elastic modulus was calculated according to Toyras and colleagues' method with an individual scaling factor for each specimen based on cartilage thickness, indenter radius, and indentation depth (15).

Statistical analysis. All data collected in this study were recorded and analysed using SPSS Statistics 26 (IBM, Armonk, NY, USA). The normality of the data was assessed by the Shapiro-Wilk test. Means for defect filling, thickness, and elastic modulus of the repair

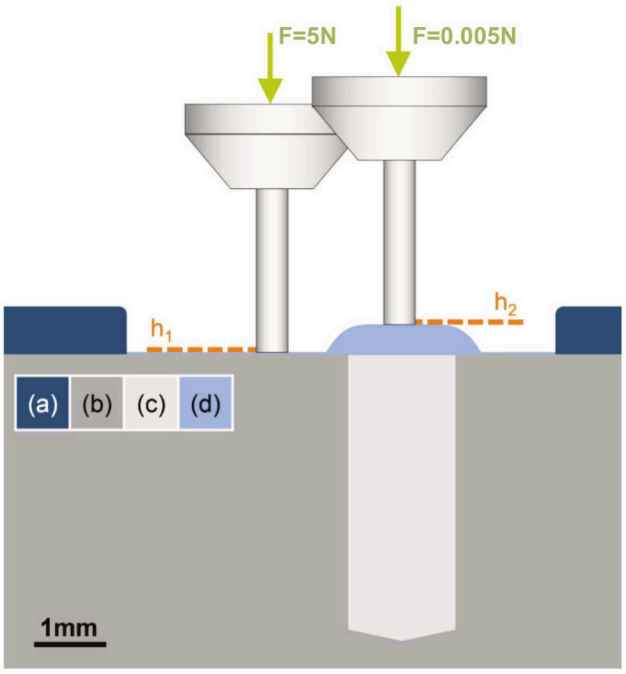

Figure 3. *Measurement of the repair cartilage thickness using a custom-built dynamic indentor test system (14): (a) native cartilage, (b) subchondral bone, (c) MFX or DRL hole, and (d) repair cartilage tissue. Repair cartilage thickness was calculated as the difference in indentor positions h_1 and h_2. MFX: Microfracturing; DRL: microdrilling.*

cartilage tissue of each treatment group were compared by one-way ANOVA and Tukey's post hoc test.

Based on previous studies, sample-size power analysis of b=0.20 and a=0.05 was performed using mean differences for defect filling between the groups. Based on this analysis and potential attrition in animal studies, a minimum of ten FCDs per group was needed to power the study adequately (11).

Results

The results for defect filling, thickness, and elastic modulus of native and repaired cartilage tissue are summarized in Table II. Quantitative assessment of defect filling showed significantly better results in all treatment groups compared to untreated FCDs in the control (p<0.001). Significant differences were also identified between the MFX and DRL treatment groups (Figure 4).

The thickness of the repair cartilage tissue was comparable throughout all treatment groups. It was slightly thinner than adjacent native cartilage tissue. A statistically significant difference could only be identified between native cartilage and the MFX1 group (p=0.023).

The elastic modulus of the repair cartilage tissue of both DRL groups, DRL1 and DRL2, was comparable to native cartilage. Both MFX groups exhibited significantly inferior

Table II. *Mean focal chondral defect (FCD) filling, repair cartilage thickness, and elastic modulus for each study group and adjacent native cartilage.*

	Defect filling (%)	Thickness (mm)	Elastic modulus (MPa)
	M (SD)	M (SD)	M (SD)
Control	45.8 (8.2)	-	-
MFX1	64.8 (13.5)	0.429 (0.210)	0.16 (0.17)
MFX2	70.7 (11.4)	0.533 (0.236)	0.17 (0.18)
DRL1	77.9 (11.7)	0.559 (0.168)	0.71 (0.39)
DRL2	84.2 (6.6)	0.490 (0.221)	0.66 (0.38)
Native cartilage	-	0.669 (0.249)	0.63 (0.34)

MFX: Microfracturing; DRL: microdrilling; M: mean; SD: standard deviation.

results for elastic modulus compared to both DRL groups (MFX1 *vs.* DRL1: p=0.001; MFX1 *vs.* DRL2: p=0.008; MFX2 *vs.* DRL1: p<0.001; MFX2 *vs.* DRL2: p=0.004) and native cartilage tissue (MFX1: p=0.002; MFX2: p<0.001) (Figure 5).

Discussion

In preclinical animal models, it is of utmost importance that the clinical picture and the treatment methods can be transferred to humans. Anatomically, Osterhoff *et al.* considered the ovine medial condyle as a suitable model because it resembles the shape of the human counterpart, and is only smaller by approximately one-third (16). Furthermore, the medial femoral condyle is the most common location of focal cartilage lesions in humans (17-21). As an indication for treatment with bone marrow-stimulating procedures in humans is seen in full-layer cartilage defects up to a size of 2 cm^2 (22-24), the defect area of 0.28 cm^2 has been chosen in the model to correspond to a frequent clinical situation in humans (16). The defect model thus fulfils the typical localization, defect area, and defect depth of cartilage defects that would have been treated by drilling or microfracturing in a clinical setting.

Another criterion to choose a certain animal model is the biological regenerative capacity of the species. The subchondral bone, which is important for cartilage regeneration, has a thicker subchondral plate in sheep than in humans (25). Nevertheless, the regenerative potential is considered comparable (26). As in the human situation, the age of the animals is also important since young animals have a higher potential to regenerate than older ones. Consequently, studies intended to reflect the human situation must be conducted in adult animals. For this reason, only

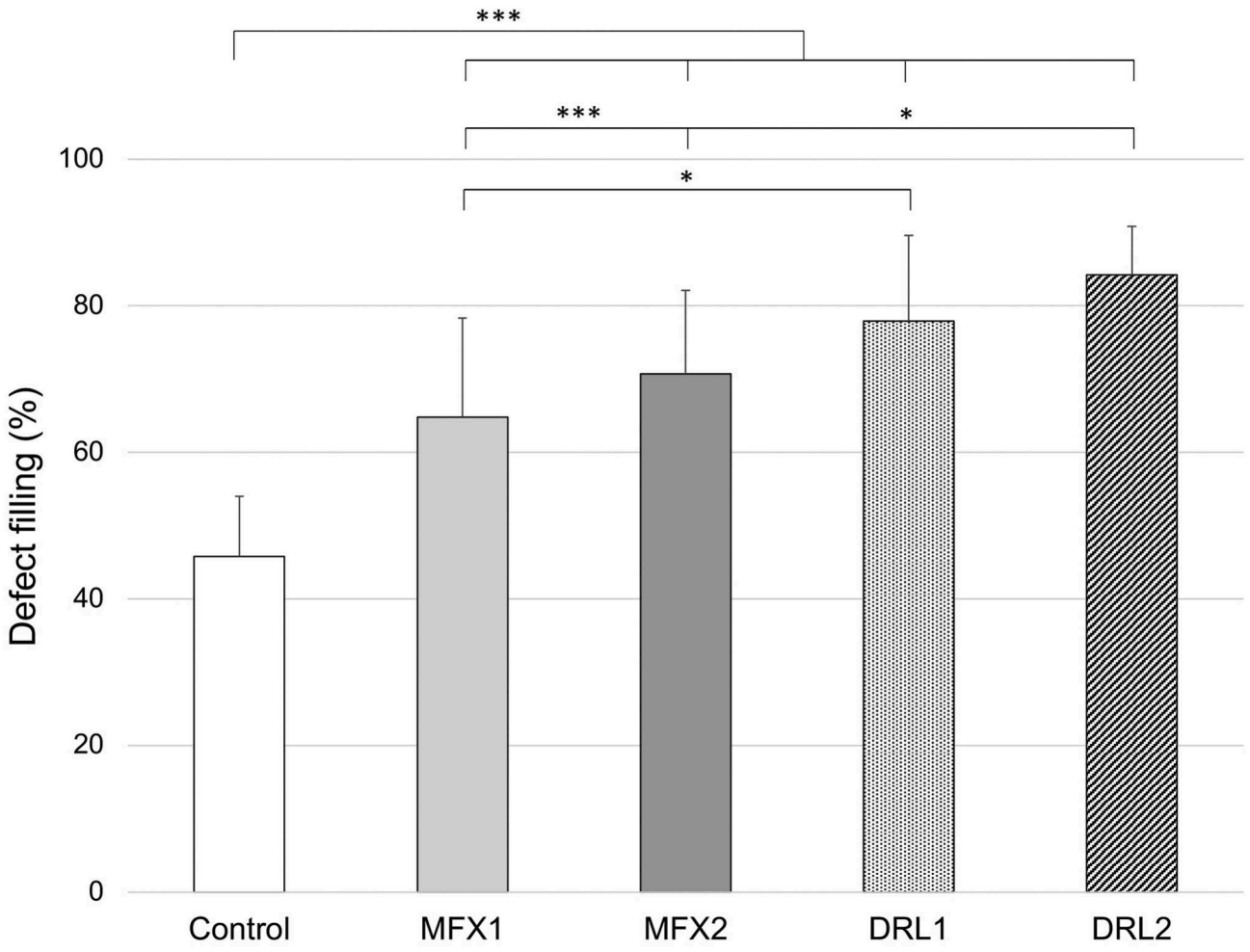

Figure 4. *Comparison of mean defect filling for all treatment groups and untreated FCDs by one-way ANOVA (error bars indicate SD); significant differences are indicated by asterisks (*p<0.05, **p<0.01, ***p<0.001). MFX: Microfracturing; DRL: microdrilling.*

adult sheep were used in the present study. After 12 months of experimental duration, cartilage regeneration and maturation should be completed, and biomechanical differences should be detected, if present (27).

Among the groups, the six-hole drilling group (DRL2) achieved the best defect filling. It was significantly higher than the filling in both microfractured groups. Quantitative defect filling was determined with ImageJ, which is widely used in the biomedical analysis of images due to its accessibility and modularity (13). While cartilage defect-filling has been subjectively classified into different grades by examiners (28), Beck *et al*. showed the feasibility of objective image analysis using ImageJ in these situations (29). Notably, drilling showed better results than microfracturing, and deeper penetration of the subchondral bone showed better results than shallower holes. Furthermore, 6 drill holes exhibited better defect filling than 3 holes for MFX and DRL. However, filling of the defect alone does not allow any conclusions about the quality and biomechanical competence of the regenerated tissue. From a mechanical point of view, however, complete filling of the defect is crucial to distribute loads homogenously.

Cartilage thickness can be measured either by optical determination, needle indentation or ultrasound (30). As the first two methods are destructive and the latter is dependent on the tide marker not necessarily present in regenerated cartilage (31), we established a protocol to measure the thickness *via* microindentation (14). With that, the measured thickness of native cartilage was in the expected range for sheep (26, 32). While cartilage thickness of the regenerates of the treatment groups, MFX and DRL, with at least 4 mm penetration depth were not significantly different from native cartilage, the group with only 2 mm penetration depth fell short.

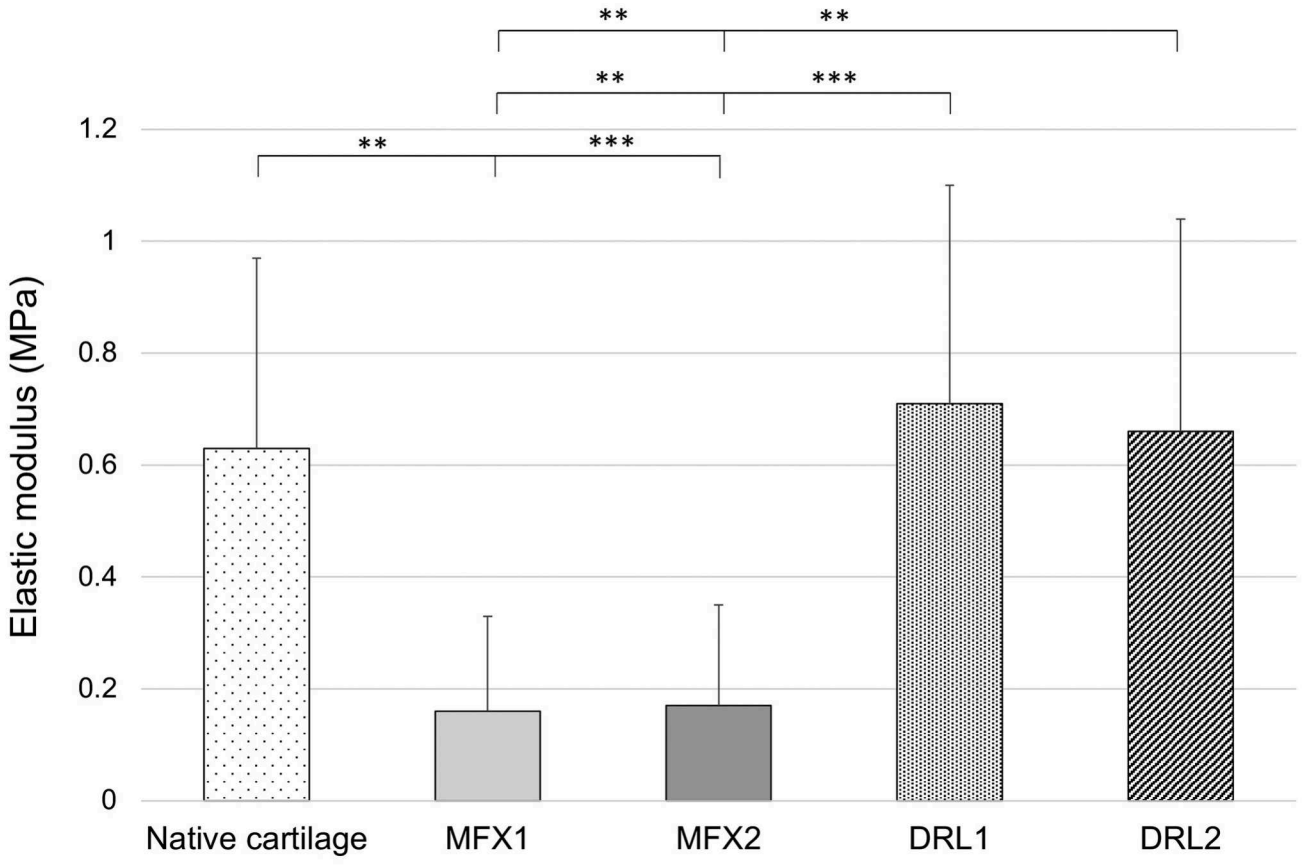

Figure 5. *Comparison of mean elastic modulus for all treatment groups and adjacent native cartilage by one-way ANOVA (error bars indicate SD); significant differences are indicated by asterisks (*p<0.05, **p<0.01, ***p<0.001). MFX: Microfracturing; DRL: microdrilling.*

There are various established methods to determine the biomechanical properties of cartilage samples *in vitro*. Compared to confined or unconfined compression, indentation is thought to have similarity to *in vivo* stress (33, 34). Our protocol is based on the work published by Töyräs *et al.* in 1999 (15), because it has proven suitable for examining cartilage tissue with altered composition and probably inferior biomechanical properties, as presented in this study. In summary, the Young's modulus of the regenerated cartilage treated with drilling showed no statistically significant difference from that of native cartilage after 12 months, regardless of the number of drill holes. In contrast, a lower Young's modulus was measured in both MFX groups, suggesting a softer regenerate. Our absolute values of Young's modulus cannot be compared to the work of Töyräs *et al.* because they used bovine cartilage and a different indentor. Nevertheless, the low modulus of the microfractured groups in the present study seems to resemble enzymatically degraded, inferior cartilage, as previously published by Töyräs *et al.* (15). Thus, differences in the opening of the subchondral bone might be crucial. As demonstrated in preclinical studies (35), drilling effectively removes bone debris and cleans margins, and the fine tunnels of the subchondral bone remain open. In contrast, microfractures densify adjacent bony structures. The cavities between the bone trabeculae are thereby sealed, which makes it difficult for mesenchymal stem cells to migrate into the defect. In addition, significantly more empty osteocytic lacunae have been observed during MFX than in DRL, which the authors attributed to direct mechanical damage of the osteocytes and shearing of the tissue (35).

To date, most knee surgeons prefer microfracturing as the favourite marrow stimulating technique, which was popularized by Steadman and is commonly seen as an enhancement/advancement of subchondral drilling proposed by Pridie in 1959 (26, 36). It is easy to perform, and there is no risk of thermal necrosis. Good to excellent clinical results can be achieved if performed for appropriate indications (37), and unfavourable outcomes might be the consequence of suboptimal mesenchymal stem cell and growth factor concentrations (38). There is no consensus about the amount and depth of subchondral perforations performed, either by

drilling or microfracture. We found better results for defect filling and biomechanical properties when drilling was performed at least 4 mm in depth and if 6 perforations were made. In the latter configuration, the mechanical properties of the regenerated cartilage were comparable to native hyaline cartilage. The worst results were achieved when performing microfracturing with only 3 holes and a depth of only 2 mm. These results contradict the current clinical practice, as most surgeons use MFX rather than DRL. It can be assumed that many surgeons still prefer the excellent availability and easier surgical technique of MFX despite the increasing evidence for better results following DRL as previously published and confirmed regarding biomechanical properties in the present study (1).

To enhance the efficacy of bone marrow stimulating techniques, synthetic and autologous biological adjuvants, including MSCs or platelet-rich plasma (PRP), have also been recently used as supplements to gain better repair tissue quality and long-term durability (39-41). However, according to our data, this improvement might also be accomplished by deeper MFX and DRL holes leading to a local release of the adjuvants from the subchondral bone. Other than previously published, no osteonecrotic lesions of the subchondral bone were detected in our animal model. However, these lesions are specific complications of these procedures and can be difficult to treat (42).

The postoperative care in our study corresponds to the usual procedures for studies in large animals. With a 12-month experimental duration, our study was exceptionally long lasting (9-11, 32, 43, 44). In contrast to clinical practice in humans, no partial weight bearing was feasible in the present experimental setting. Thus, cartilage quality and defect filling might be even better in a human clinical setting.

However, some limitations of the present study must be noted. First, despite comparable anatomic morphology, differences between the ovine and human knee exist. Furthermore, no partial weight bearing can be achieved in an animal model. Both aforementioned aspects may limit direct transferability to the clinical situation in humans. Second, open surgery was performed in the present study, in which drilling small holes is technically easy and a drill template could be used. However, implementation of DRL in a clinical arthroscopy setting can be challenging, and arthroscopic drill templates would be necessary to ensure evenly distributed holes. Third, no biomechanical testing of shear forces was performed to confirm the excellent results of the DRL groups obtained during elastic modulus testing.

Conclusion

In the present study, an ovine model, which is comparable to the clinical situation in humans, was used to compare MFX and DRL regarding formation and biomechanical properties of repair cartilage tissue in critical size FCDs of the knee. Overall, more defect filling and better biomechanical properties of the repair cartilage tissue were identified for DRL compared to MFX. DRL with 6 holes and a penetration depth of 4 mm led to the highest level of defect filling and the repair cartilage tissue exhibited biomechanical properties that were comparable to native hyaline cartilage. These findings are in contrast to current clinical practice with MFX as the gold standard and suggest a clinical return to DRL.

Conflicts of Interest

All Authors declare that they have no conflicts of interest.

Authors' Contributions

Florian Pohlig, MD, Ph.D.: conducted experiments, data analysis, preparation of manuscript, approval of manuscript. Michael Wittek, MD: conducted experiments, data analysis. Anne von Thaden, MD: conducted experiments, preparation of manuscript, approval of manuscript. Ulrich Lenze, MD, Ph.D.: data analysis, preparation of manuscript. Claudio Glowalla, MD: data analysis, preparation of manuscript. Philipp Minzlaff, MD, PhD: preparation of manuscript, approval of manuscript. Rainer Burgkart, MD, PhD: study design, approval of manuscript. Peter Michael Prodinger, MD, Ph.D.: conducted experiments, data analysis, preparation of manuscript, approval of manuscript.

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