No Effect of Hole Geometry in Microfracture for Talar Osteochondral Defects

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Abstract

Background Débridement and bone marrow stimulation is an effective treatment option for patients with talar osteochondral defects. However, whether surgical factors

affect the success of microfracture treatment of talar osteochondral defects is not well characterized.

Questions/purposes We hypothesized (1) holes that reach deeper into the bone marrow-filled trabecular bone allow for more hyaline-like repair; and (2) a larger number of holes with a smaller diameter result in more solid integration of the repair tissue, less need for new bone formation, and higher fill of the defect.

Methods Talar osteochondral defects that were 6 mm in diameter were drilled bilaterally in 16 goats (32 samples). In eight goats, one defect was treated by drilling six 0.45-mm diameter holes in the defect 2 mm deep; in the remaining eight goats, six 0.45-mm diameter holes were punctured to a depth of 4 mm. All contralateral defects were treated with three 1.1-mm diameter holes 3 mm deep, mimicking the clinical situation, as internal controls. After 24 weeks, histologic analyses were performed using Masson-Goldner/Safranin-O sections scored using a modified O'Driscoll histologic score (scale, 0–22) and analyzed for osteoid deposition. Before histology, repair tissue quality and defect fill were assessed by calculating the

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The authors certify that the animal protocol for this investigation was approved by the Academic Medical Center and VU University, Amsterdam, and that all investigations were conducted in conformity with ethical principles of research.

The design and creation of surgical templates was performed at TU Delft, Delft, The Netherlands. Animal operations were performed at VU University, Amsterdam, The Netherlands. Histology was performed at ACTA, Amsterdam, The Netherlands. Micro-CT acquisition and analyses were performed at Erasmus MC, Rotterdam, The Netherlands. Analyses were performed at Academic Medical Center, Amsterdam, The Netherlands.

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mean attenuation repair/healthy cartilage ratio on Equilibrium Partitioning of an Ionic Contrast agent (EPIC) micro-CT (μ CT) scans. Differences were analyzed by paired comparison and Mann-Whitney U tests.

Results Significant differences were not present between the 2-mm and 4-mm deep hole groups for the median O'Driscoll score ($p = 0.31$) and the median of the μ CT attenuation repair/healthy cartilage ratios ($p = 0.61$), nor between the 0.45-mm diameter and the 1.1-mm diameter holes in defect fill ($p = 0.33$), osteoid ($p = 0.89$), or structural integrity ($p = 0.80$).

Conclusions The results indicate that the geometry of microfracture holes does not influence cartilage healing in the caprine talus.

Clinical Relevance Bone marrow stimulation technique does not appear to be improved by changing the depth or diameter of the holes.

Introduction

Débridement and bone marrow stimulation is a simple, cost-effective operative treatment for osteochondral defects with lower morbidity and faster return to activity than open cartilage restoration surgery [6, 11, 12, 39, 45, 50]. Systematic reviews have shown a current clinical success percentage of 86%. The affect of patient and defect-specific prognostic factors such as lesion size [11, 12], location [11, 40], age [11, 20, 43], or body weight [12, 18] is inconclusive owing to the absence of well-designed prospective studies [18, 19]. The exact mechanisms of the healing process are unknown, which makes prediction of clinical outcome difficult [36, 48].

There is little, and only experimental, research on whether improvements in surgical technique can enhance the healing process of damaged cartilage surfaces. One animal study suggests that the depth of subchondral perforation influences the degree of fill and quality of repair tissue [7]. Other research indicates that there is a difference in bone structure and degree of bone necrosis between drilling and microfracture [8, 29].

A recent systematic literature review showed a large degree of similarity in current surgical techniques for microfracture [19], including use of K-wires [2, 22, 31] or awls [43], removal of unstable cartilage, hole depth between 2 and 4 mm until bleeding or fat droplets occur, and hole spread with a distance of 3 to 4 mm. These recommendations are similar to the originally presented technique by Steadman et al. [43]. Additional recommendations are creation of a stable rim, placement of the holes perpendicular to the surface, and removal of the calcified layer at the base of the defect [9, 13, 23]. To the best of our knowledge, there are no published studies regarding

whether hole size or distance between the defects influences the degree and quality of the repair tissue.

The aim of our study was to determine the influence of hole geometry, when performing microfracture treatment in the talus, on the quality of the repair cartilage and the filling grade of the defect in a goat model. We formulated two hypotheses: (1) holes that reach deeper into the bone marrow-filled trabecular bone result in better quality repair tissue; and (2) a larger number of holes with a smaller diameter result in a more solid integration of the repair tissue, less need for new bone formation, and higher fill of the defect.

Materials and Methods

Sixteen female Dutch milk goats (*Capra hircus sana*), 4 years of age, with an average weight of 69 kg (range 44–86 kg), were used in this study. Screening for pregnancy and disease was performed before entering the trial. The goats were kept in group housing starting 1 week before and continuing until 1 week after surgery to minimize stress. The number of goats was determined from a sample size calculation performed using a power of 90% and two-sided significance level of 5% between groups. A 10% SD of the histology score was described by O'Driscoll et al. [28]; therefore, a minimal effect of 15% was considered clinically significant [4, 21]. Our study protocol was approved by the local animal welfare committees (ORCA102287).

Talar defects are predominantly osteochondral defects, unlike the knee where chondral defects most often are seen [3, 9, 11, 15, 47]. The primary surgical treatment for both is microfracture [45, 50]. To best represent the clinical situation we created an osteochondral talar defect. The dimensions of the created defect depth and diameter and the distance between the microfracture holes in this study were scaled down using the respective ratio between a critical-size osteochondral defect in the human (15 mm diameter) and in the smaller goat talus (6 mm diameter) [9, 17]. Therefore, defect depth was 3 mm, reaching just underneath the subchondral bone plate [10] but sufficiently shallow to avoid preemptive spontaneous bleeding (Fig. 1). One defect in each goat served as a reference mimicking standard clinical microfracture dimensions: three holes of 1.1 mm diameter and 3 mm deep (Table 1). The contralateral defect served to test our hypotheses. To investigate the effect of microfracture hole depth, this defect was treated with microfracture awls penetrating only the subchondral bone plate (2 mm) in half of the goats, whereas in the other half, the holes reached the center of the talus (4 mm) (Table 1). To determine the effect of the microfracture hole diameter and number, the contralateral defects of the goats were treated with six holes

Fig. 1A–E A 6-mm diameter osteochondral defect was drilled in the talus of both hind legs. In the same session, one defect subsequently was treated with six 0.45-mm diameter puncture holes of either 2 mm or 4 mm depth. The contralateral defect was treated with three puncture holes made by a 0.045- (1.1 mm diameter) K-wire capped to a depth of 3 mm to simulate the dimensions of the current clinically applied microfracture awls. (A) The customized drill guide was

designed to create a 3-mm deep defect. (B) The microfracture tools used to standardize the diameter and distance between the holes are shown. (C) The 6-mm diameter, 3-mm depth created defect was made without bleeding from the defect before treatment. (D) The defect was treated with six 0.45-mm diameter holes with punctuate bleeding from the holes. (E) The contralateral defect was treated with three holes of 0.045 K-wires (1.1 mm diameter).

Table 1. Treatment group specifications

Number of specimens	Talus	Number of holes	Diameter	Depth
8 goats (16 defects)	Randomized talus (8 defects)	6	0.45 mm	2 mm
	Contralateral talus (8 defects)	3	1.1 mm	3 mm
8 goats (16 defects)	Randomized talus (8 defects)	6	0.45 mm	4 mm
	Contralateral talus (8 defects)	3	1.1 mm	3 mm

of 0.45-mm diameter, keeping the total treatment surface area constant (Table 1).

All operative procedures were performed in a standardized manner by the first author (ACK), one experienced orthopaedic surgeon (GMMJK), and an assistant (Appendix 1).

A posterolateral surgical approach was used to access the talus [46]. Through a 6-mm diameter cannulated drill guide, the osteochondral defect was drilled in the tali of both hind legs perpendicular to the talar surface under continuous cooling with saline. The defect was placed at the center of the talar dome as designated by one surgeon for all goats. After débridement, the goats were treated according to a randomization scheme (Table 1; Fig. 1) using custom-made surgical templates. The joint capsule and subcutaneous tissue were closed with interrupted 2-0 absorbable sutures and the skin was closed with an absorbable continuous intracutaneous suture. The animals were encouraged to perform immediate weightbearing and were transferred to a farm off-site after primary wound closure to complete followup under daily observation with neither food nor exercise restriction.

After 24 weeks, the goats were euthanized. All tali were collected, photographed, and stored at 4° C in phosphate-buffered saline 1% with aspartic, serine, and

cysteine proteases inhibitors (cOmplete ULTRA Mini Tablets; Roche Diagnostics Corporation, Indianapolis, IN, USA).

To compare the microscopic quality of the repair tissue between the 2-mm and the 4-mm groups, histologic analysis was performed. Second, osteoid formation and repair tissue integration were compared in the 1.1-mm and 0.45-mm diameter groups to establish any differences in integration and bone formation between these groups. All tali were cut into 20- × 20-mm blocks around the defect, leaving the entire depth of the talus intact. The blocks were fixed in 4% formaldehyde in a 0.1 mol/L phosphate buffer, dehydrated, and embedded in methacrylate. At a quarter into and at the center of the defect, 5-μm slices were sectioned and stained alternately with hematoxylin and eosin, Safranin-O, and Masson-Goldner trichrome. Collagen fibers were examined using polarized light to assess the structure of the repair tissue. Hyaline cartilage will show organization of the fibers, whereas fibrous tissue does not.

To quantify our cartilage quality assessment, a modified form of the score described by O'Driscoll et al. was used (Appendix 2) [28]. The original score by O'Driscoll et al. contains four main categories: nature of predominant tissue, structural characteristics, freedom from cellular changes of degeneration, and freedom from degenerative changes of the adjacent cartilage, which give a maximum total score of 24 points. The subitem cartilage thickness was not scored, because the repair tissue in the defects was never thinner than the healthy cartilage surrounding the defects. Therefore, a maximum of 22 points could be scored in this study. Each sample was scored twice with a 1-month interval by one blinded observer (ACK). Differences between the scores at the two times were resolved by an experienced histologist (VE). The entire defect was represented by an average O'Driscoll score, which was

calculated using the values at a quarter and at the center of the defect.

To quantitatively assess glycosaminoglycan (GAG) content [32], sections from the center of the defect were stained with Safranin-O without any counterstaining and digitally analyzed using a Leica microscope (Leica, Wetzlar, Germany) with a 518-nm wavelength filter and evaluated using imaging software [35]. The normalized average Safranin-O intensity (SOI) ratio of the repair tissue compared with the healthy reference cartilage region in the same sections then was calculated. A ratio close to one suggests a cartilage quality of the repair tissue close to that of healthy cartilage.

Osteoid formation was assessed on regular slices using the Masson-Goldner sections from the center and categorized into three groups: (1) no osteoid formation; (2) slight, less than 20% of the defect; or (3) substantial osteoid formation, greater than 20% of the defect.

Repair tissue integrity was quantified by a subitem of the O'Driscoll score into three categories: (1) full integration; (2) slight disruptions including cysts; or (3) severe disruptions.

To compare the quality of the repair tissue between the 2-mm and 4-mm deep groups, a noncontrast-enhanced micro-CT scan (μ CT) and an equilibrium partitioning of an ionic contrast agent (EPIC) through μ CT (EPIC- μ CT) scan were acquired of each entire talus using a μ CT scanner (Skyscan1076; Skyscan, Kontich, Belgium) [30, 49] before histologic analysis. The EPIC- μ CT was acquired after the tali had been saturated for 24 hours in a 40% Hexabrix 360 dilution (Guebet BV, Gorinchem, The Netherlands) [49]. The following scan settings were used: isotropic voxel size of 18 μ m; voltage of 70 kV; current of 111 μ A; 0.5-mm aluminium filter; and 198° with a 0.4° rotation step. Using Skyscan analysis software (Skyscan), a normalized attenuation (gray value) ratio was calculated between a region of cartilage repair tissue and a region of healthy cartilage on the opposite side of the talus. A normalized attenuation ratio close to one indicates that the quality of the repair tissue is close to that of healthy cartilage.

To compare the effect on repair tissue volume between the 4-mm and 2-mm deep holes the cartilage repair tissue volume was determined as a percentage of the total tissue volume of a three-dimensional volume of 8 × 8 × 8-mm around the center of the defect. Two observers (ACK, SdD) individually determined the repair tissue volume using image software [35], and disagreements between the observers were solved by discussion resulting in a single value per sample (Fig. 2).

All surgical wounds healed without infection. Two complications occurred after surgery: one temporary pressure neuropathy of the peroneal nerve, which subsided completely within 12 hours, and one persistent

swelling of both hind legs causing impeded mobilization. Supportive treatment was given using cooling gel. One goat was terminated early at 22 weeks followup for a bilateral front hoof problem causing walking difficulty and pain. There were no apparent problems with the hind legs. The last 2 weeks at the end of the followup period were expected to have had minimal influence on the overall healing tendency. Therefore, these tali were included for analysis. All other 15 goats completed followup without difficulty.

The modified O'Driscoll scores including subitems, attenuation ratios, and repair tissue volume percentages from the EPIC-CT, the Safranin-O intensity ratios, and the osteoid formation categories were analyzed nonparametrically as a result of skewed distribution of the data and the small sample sizes. Paired analysis between the experimental and control sides of each goat was performed to decrease the influence of repair variability between the goats. In addition, the differences between the talus treated with the experimental treatment and the contralateral talus were calculated per goat using Mann-Whitney U tests ($p \leq 0.05$). The EPIC- μ CT attenuation ratios correlated significantly with the SOI ratio for the experimental groups ($r^2 = 0.5$; $p = 0.05$) and the control defects ($r^2 = 0.7$; $p = 0.006$). In one μ CT of the 2-mm group, an artifact was seen on the defect. This sample and its matching control were excluded for this specific analysis.

Results

We found no clinically significant relationship between repair tissue quality and the depth of microfracture holes. The median difference in the modified O'Driscoll score between the treatment and control legs per goat per group was 1.3 (range, -1.3 to 4.3) for the 2-mm group and -0.9 (range, -4.3 to 2.0) for the 4-mm group (Table 2). This difference was statistically significant ($p = 0.04$). All sections contained predominantly fibrous cartilage repair tissue with diminished Safranin-O staining and disorganized collagen structures on polarized light microscopy. The majority of the defect surfaces showed smooth surfaces (18 samples; Fig. 3). One goat in the 2-mm group had large residual defects with a cleft in the center in both tali (Fig. 3). No cause could be identified from the operation or the postoperative period. Thirteen samples showed progressive hypocellularity near the surface in a zonal distribution compared with deeper zones near the subchondral bone plate (six controls, three in the 2-mm group and four in the 4-mm group; Fig. 3). Digital analysis of the Safranin-O intensity showed five samples in the control group and two in the 4-mm experimental group with uniform Safranin-O distribution with moderate staining. The

Fig. 2A–C (A) A photograph of the talus with the treated defect is shown. The schematic representations of the same talus in the (B) cranial and (C) lateral views show the two locations of histologic

analysis (dotted lines) and the $8 \times 8 \times 8$ -mm region of interest in the μ CT of which the percentage of repair tissue area was calculated.

Table 2. Results per depth group

Depth	2 mm	Corresponding control	Difference	4 mm	Corresponding control	Difference	Difference between groups
Modified O'Driscoll score	12.5	12.0	1.3	11.5	12.6	−0.9	2.4 [†]
Median (range)	(10.8–15.3)	(9.0–13.0)	(−1.25 to 4.3)	(9.8–16.0)	(11.5–14.3)	(−4.3 to 2.0)	($p = 0.04$)
Safranin-O intensity ratio	1.8	1.7	0.2	1.5	1.4	0.2	0
Median (range)	(1.4–2.7)	(1.1–4.7)	(−3.0 to 1.3)	(0.9–2.4)	(1.0–2.3)	(−1.2 to 0.5)	(NS)
Micro-CT attenuation ratio	1.6	1.6	0.01	1.6	1.5	0.03	0.02
Median (range)	(1.5–1.8)	(1.4–1.7)	(−0.2 to 0.4)	(1.3–2.1)	(1.3–1.8)	(0.3–0.8)	(NS)

[†]Difference between 2-mm and 4-mm groups is significant ($p = 0.04$); NS = not significant ($p > 0.05$).

other defects showed only patches of staining (Fig. 3). The difference in SOI ratio per goat per group was the same, 0.2, for the 2-mm and the 4-mm groups. This was not significant (Table 2). No significant difference was present between the attenuation ratio of the 2-mm group (range, −0.2 to 0.4) or the 4-mm group (range, −0.0 to 0.8) (Table 2). In all scans the repair tissue was largely saturated with the contrast agent (average grey value 84/255, 33%) indicating a relatively low GAG content, compared with the relatively low saturation (average grey value 53/255, 21%), indicating high GAG content for the healthy adjacent cartilage (Fig. 4).

We also found no relationship between defect fill or repair tissue integrity between the 2-mm and 4-mm groups. On histology, the O'Driscoll score subitem “structural integrity” was not significantly different between the 2-mm and 4-mm groups nor was the osteoid formation (Table 3). Six samples showed severe disruption of tissue integrity, evenly distributed over both experimental groups and the control groups (Fig. 3). No significant difference was present in repair tissue volume on the EPIC-CT scans (range, 10%–75%; Table 3).

Discussion

The aim of our study was to determine the effect of depth and diameter of holes created with microfracture bone marrow stimulation on repair tissue quality and the filling grade of the defect. We found that hole depth and hole diameter did not influence the defect fill or the structural integrity of repair tissue. To our knowledge, there are no previous comparative studies that have investigated hole diameter to compare with these results.

Our study has some limitations. Despite the efforts to standardize the protocol, a large variation was present in quality of repair tissue and defect fill even though we used a sample size calculation that was based on previous studies [4, 21, 27, 46]. The variation might be because the goats were not restricted in mobilization causing some goats to mobilize at a different pace [1]. However, signs of disturbed healing resulting from too fast or hampered mobilization (eg, limping, swelling) were not seen. In addition, there was a large range in weight in our animals (range, 47–90 kg). However, this proved to be attributable to two outliers, whereas 14 of 16 goats weighed 60 to 80 kg. The results of

Fig. 3A–L An example of a best case result is shown with (A) moderate Safranin-O staining with evenly distributed GAG (original magnification, $\times 400$), (B) Masson-Goldner staining with little osteoid formation (original magnification, $\times 200$); and (C) Safranin-O without counterstains used for digital Safranin-O intensity with moderate staining and evenly distributed GAGs, and a smooth surface without disruptions or cysts in the repair tissue. An example of a moderate result is shown with (D) Safranin-O staining with patches of moderate staining and also unstained tissue (original magnification, $\times 400$); (E) Masson-Goldner staining with several osteoid isles visible underneath the repair tissue (black arrows) (original magnification, $\times 200$); and (F) Safranin-O without counterstains showing patches of moderate staining and also unstained tissue, are shown. Overall, the staining is less than the healthy tissue (*repair tissue, white arrow = adjacent cartilage), and the repair tissue has not bonded to the healthy tissue

(black arrow). An example of a worst case result is shown with (G) Safranin-O staining with a strong zonal distribution of Safranin-O and an irregular surface (original magnification, $\times 400$); (H) Masson-Goldner staining with large osteoid formations (original magnification, $\times 200$); and (I) Safranin-O without counterstains used for digital Safranin-O intensity (SOI), with a large difference in SOI between the repair tissue staining are shown (*repair tissue; white arrow = adjacent cartilage). The repair tissue has not bonded to the adjacent cartilage (black arrow). (J) Safranin-O staining of a sample with a cleft in the center shows similar results to the moderate case with moderate Safranin-O staining (original magnification, $\times 400$). (K) A few osteoid isles can be seen on the sample with Masson-Goldner staining (original magnification, $\times 200$). (L) There was uneven Safranin-O distribution and detachment of the healthy cartilage on the Safranin-O without counterstains digital analysis.

Fig. 4A–B Two samples of EPIC- μ CT images are shown. The arrows indicate the repair tissue site in the cartilage. **(A)** The contrast influx is close to that of the adjacent cartilage, indicating a relatively similar GAG content. This results in an attenuation ratio (repair

tissue: healthy reference) close to 1. **(B)** In this sample the attenuation ratio is lower owing to the low attenuation in the repair tissue caused by the lower negative charge density.

Table 3. Results per diameter group

Diameter	0.45 mm	1.1 mm	Difference
Structural integrity (O'Driscoll score)			
Normal	1 (6%)	4 (25%)	NS
Slight disruption	10 (63%)	9 (36%)	
Severe disruption	5 (31%)	3 (19%)	
Osteoid formation			
None	1 (6%)	2 (13%)	NS
Moderate	8 (50%)	8 (50%)	
Severe	7 (44%)	6 (38%)	
Repair tissue volume			
Median (range)	23% (10%–40%)	29% (10%–75%)	–3% (NS)

NS = not significant ($p > 0.05$).

the two outliers were similar to those of the other goats. We do not expect this to have influenced our results, because the literature is controversial regarding the effect of weight on the outcome of microfracture [5, 19, 23]. Another limitation is the use of the smaller joint (the ankle) rather than the knee. This limited the extremes in microfracture hole depth between the treatment groups in the current study. All other animal studies used the knee [8, 17, 24, 41, 42]. The maximum depth for the microfracture holes in the talus was 4 mm when using a 3-mm deep osteochondral defect and without penetrating the center of the talus. Other studies used depths between 2 and 6 mm [7, 8, 24]. In addition, the knee is connected to a shaft bone containing a large reserve of bone marrow [33], whereas the talus is dependent on the external vascular supply and the cells present in the trabecular bone [34]. These differences might have limited the number of cells that could be recruited from deeper areas. However, the talus is a more congruent joint and such a confined space might protect the blood clot from detaching during initial weightbearing. Immediate weightbearing on the defects also could have enhanced

repair response compared with nonweightbearing locations (eg, the trochlea), which correspond to less hyaline repair tissue [16]. Additionally, talar cartilage is known to be stiffer and denser with a higher GAG and lower water content [44]. This changes the physical properties of the cartilage and its response to cyclic loading. Further clinical research is needed to investigate whether the biomechanical differences also might influence the healing response, clinical outcome, and controversial prognostic factors in the two most predominantly used joints for bone marrow stimulation [25, 41]. This should be taken into account when applying our results to the knee, especially because we used an animal model to answer our research question. Extrapolating results from an animal study to a clinical situation should be done cautiously. In many cartilage studies smaller, mainly rabbit models have been used [6, 8, 10, 41]. We chose a goat model because this allows creation of larger defects (≥ 6 mm in diameter) that do not heal spontaneously [17]. In addition, the cortical and trabecular bone structure of the goat is similar to human bone and the proportion of cartilage to subchondral bone [10, 42]. In addition, some studies had a shorter followup (range, 1–56 days) [7, 8, 42], which is less analogous to the clinical situation. Clinical studies of the ankle use a followup period that generally ranges from several months to years [5, 9, 11, 14, 22, 23, 25, 39, 50].

Concerning our first hypothesis, the differences we observed in terms of the O'Driscoll score for cartilage repair were on the border of statistical significance favoring 2-mm deep holes over 4-mm deep holes, but did not reach the threshold of 15% defined elsewhere as clinically significant [38, 42]. A study using a rabbit model indicated that deeper compared with shallower drilling (6 mm versus 2 mm) elicited a cartilage repair tissue with a more hyaline character in the repair matrix [7]. We did not find this in our study. The predominantly fibrous repair tissue that we observed was similar to that in other cartilage repair studies using the microfracture technique [6, 24, 50]. This also caused a considerable

saturation level of the repair tissue in the EPIC- μ CT of the repair tissue, resulting from the relatively low GAG content. More subtle zonal differences in the repair tissue as seen in the Safranin-O slices remained undetected. Further research is needed to determine the right saturation protocol of fibrous tissue to enhance the potential of EPIC- μ CT analysis of cartilage repair tissue.

Concerning our second hypothesis, no differences were found between 0.45-mm diameter microfracture holes and 1.1-mm diameter holes in the “structural integrity” sub-item of the O’Driscoll score, repair tissue volume, or osteoid formation (Table 3). Another study also used the O’Driscoll score to assess 6-mm diameter defects in goats treated with subchondral drilling (mean score, 11.3; SD, 8.7) [24]. A couple studies reported limited bonding to the subchondral bone and adjacent cartilage and only 30% to 50% defect fill [6, 24]. The modified O’Driscoll score in our study was higher and all samples showed good bonding to the subchondral bone, bonding to the adjacent cartilage on at least one side, and higher overall defect fill (Fig. 3; Table 3).

The study has several strengths. First, a high level of standardization was achieved by the use of specifically designed surgical templates to minimize variation in hole diameter, depth, and hole dispersion. Second, the minimally invasive approach decreased the operative burden on the animal and reduced the chance of postoperative complications. Third, the animals were used as their own controls to compensate for the high variability between goats. A control group with an untreated defect was deemed an unnecessary increase of used animals, because previous studies have shown the significant lack of healing of these defects [24, 46]. Fourth, samples were analyzed according to the guidelines for cartilage assessment in animal studies [16]. For cartilage assessment, the modified O’Driscoll score was used [28] because it is validated in animal models [26, 37]. Supporting the modified O’Driscoll score, two semiautomatic quantitative techniques were used as an internal reference: μ CT and digital Safranin-O analysis. The use of μ CT also offered a three-dimensional volume in which bone and cartilage repair could be assessed in the entire defect. Using these tools and approaches, we found no clinically significant difference in the cartilage repair tissue quality or degree of defect fill of talar osteochondral defects treated with different sized microfracture holes or holes with different depths.

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Appendix 1

Substances used during operative treatment and euthanasia

1. General anesthesia

10 mg/kg ketamine (Alfasan International BV, Woerden, The Netherlands)

1.5 mg atropine (Centrafarm Services BV, Etten-Leur, The Netherlands)

10–20 mg etomidate intravenously (B. Braun Melsungen AG, Melsungen, Germany) on effect per goat

250 μ fentanyl bolus intravenous injection (Hameln pharmaceuticals gmbh, Hameln, Germany) and repetition based on heart rate

15 mg midazolam bolus injection intravenously (Dormicum; Roche Nederland BV, Woerden, The Netherlands) and repetition on heart rate

1%–2.0% isoflurane (Nicholas Piramal Limited, London, UK) per inhalation

2. Epidural injection

0.1 mg/kg morphine in 4 mL NaCl 0.9%

3. Postoperative pain medication

One 75- μ g/hour Duragesic® patch (Janssen Cilag BV, Tilburg, The Netherlands)

0.02 mg/kg meloxicam subcutaneously once daily, 5 days maximum

4. Euthanasia

10 mg/kg ketamine (Alfasan International BV)

1.5 mg atropine (Centrafarm Services BV)

10 mg xylazine intramuscularly (Sedazine®; ASTfarma, Oudewater, The Netherlands)

20 mg/kg pentobarbital sodium intravenously (Euthasol 20%; ASTfarma)

Appendix 2

Summary and dispersion of the modified score of O’Driscoll et al.

Modified O’Driscoll score		
Characteristics	Categories	Points
Nature of predominant tissue morphology	Hyaline articular cartilage	4
	Incompletely differentiated mesenchyme	2
	Fibrous tissue or bone	0

Appendix 2. continued

Modified O'Driscoll score

Characteristics	Categories	Points
Safranin-O staining of the matrix	Normal or nearly normal	3
	Moderate	2
	Slight	1
	None	0
Structural characteristics*		
Surface regularity	Smooth and intact	3
	Superficial horizontal lamination	2
	Fissures 25% to 100% of the thickness	1
	Severe disruption, including fibrillation	0
Structural integrity	Normal	2
	Slight disruption, including cysts	1
	Severe disintegration	0
Bonding to the adjacent cartilage	Bonded at both ends of graft	2
	Bonded at one end, or partially at both ends	1
	Not bonded	0
Freedom from cellular changes of degeneration		
Hypocellularity	Normal cellularity	3
	Slight hypocellularity	2
	Moderate hypocellularity	1
	Severe hypocellularity	0
Chondrocyte clustering	No clusters	2
	Less than 25% of the cells	1
	25% to 100% of the cells	0
Freedom from degenerative changes in adjacent cartilage		
	Normal cellularity, no clusters, normal staining	3
	Normal cellularity, mild clusters, moderate staining	2
	Mild or moderate hypocellularity, slight staining	1
	Severe hypocellularity, poor or no staining	0

* The subitem "Thickness of repair tissue" was left out because all repair tissue was always thicker than the adjacent cartilage and therefore could not be scored accurately. (Modified score of O'Driscoll et al. published with permission from O'Driscoll SW. Adapted from Table II in O'Driscoll SW, Keeley FW, Salter RB. Durability of regenerated articular cartilage produced by free autogenous periosteal grafts in major full-thickness defects in joint surfaces under the influence of continuous passive motion: a follow-up report at one year. *J Bone Joint Surg Am.* 1988;70:595–606.)

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