

Cartilage repair in the rabbit knee: mosaic plasty resulted in higher degree of tissue filling but affected subchondral bone more than microfracture technique

A blinded, randomized, controlled, long-term follow-up trial in 88 knees

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Abstract

Purpose Discrepancies and variances in outcome following different surgical techniques for cartilage repair are poorly understood. Successful repair relies on proper tissue filling without initiating degenerative processes in the cartilage–bone unit. Consequently, the objective of the current study was to compare two available techniques for cartilage repair, i.e., microfracture technique and mosaic plasty, regarding tissue filling and subchondral bone changes in an experimental model.

Methods A 4-mm pure chondral defect was created in the medial femoral condyle of both knees in New Zealand rabbits, aged 22 weeks. A stereomicroscope was used to optimize the preparation of the defects. In one knee (randomized), the defect was treated with microfracture technique whereas in the other with mosaic plasty. The animals were killed at 12, 24 and 36 weeks after surgery. Defect

filling, new bone formation above the level of the tidemark and the density of subchondral mineralized tissue were estimated by histomorphometry.

Results Mosaic plasty resulted in a significantly 34% higher degree of tissue filling than microfracture technique at 36 weeks, SD of mean difference being 34%. Mosaic plasty resulted in significantly more new bone formation and reduced subchondral mineralized tissue density compared to microfracture technique. The differences between the two techniques were apparent mainly at the long-term follow-up.

Conclusion Tissue filling is a limiting factor regarding microfracture technique when compared to mosaic plasty, whereas mosaic plasty resulted in more bone changes than microfracture technique—the implications of the latter remain to be settled. This study underlines the difficulty in predicting outcome in the single case with any of these two techniques, particularly in a long-term perspective.

Level of evidence II.

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Keywords Cartilage · Chondral defect · Microfracture · Mosaic · Filling · Subchondral bone · Knee · Rabbit · Surgery

Introduction

Focal articular cartilage injuries in the knee are common [3, 30], and the complaints they cause may impair quality of life as much as severe osteoarthritis [29]. Injured articular cartilage has a limited capacity for complete spontaneous healing [12], and a number of therapeutic measures have been published [9, 27, 48, 66]. However, no treatment modality has so far proved to be superior compared to the others [5, 6, 21, 23, 24, 32, 38, 39, 49, 52, 62, 67, 70], and

the results of each technique demonstrate large variances [37]. Two inexpensive and commonly used techniques for cartilage repair reported from clinical practice are the microfracture technique and mosaic plasty [27, 66]. Although each of them has been studied in separate animal models [19, 26], their discrepancies have not been evaluated by randomized comparison in standardized animal models. The amount of tissue filling reported from experimental microfracture studies varies [7, 16, 19, 20], whereas from previous experimental mosaic plasty studies, tissue filling has not been reported. Moreover, the majority of experimental studies concerning these two techniques for cartilage repair are on defects penetrating into the subchondral mineralized tissues [19, 20, 26, 31, 41, 58] corresponding to ICRS grade 3c or grade 4 defects [11]. Access to bone marrow elements might be one of the strongest predictive factors for filling of the defect itself—thus being a bias in evaluating other factors [34]. In addition, iatrogen damage to the calcified layer may cause moderate-to-severe subchondral bone loss at a long-term follow-up [8] and new bone formation above the level of the tidemark [17]. The consequences of such changes regarding cartilage repair are not clear, but abnormalities in subchondral mineralized tissue remodeling and bony advancement above the level of tidemark are associated with the progression of degenerative joint disease [14].

The primary purpose of the current study was to test the hypothesis that the choice of microfracture vs mosaic plasty would influence on the amount of filling of a chondral defect not penetrating the subchondral mineralized tissues. Secondly, the purpose was to evaluate whether the choice of cartilage repair technique would influence on new bone formation and changes in the subchondral mineralized tissues.

Materials and methods

Design/animal model

A previous established experimental animal model was used [2]. Adult New Zealand rabbits were included in a randomized study where circular lesions, diameter 4 mm, were created in the medial femoral condyle (MFC) of both knees. The lesions were pure chondral, avoiding damage to the subchondral mineralized tissues, corresponding to defects prior applied in experimental models [7, 8, 10, 22, 28, 41]. By random, the defect in one of the knees was treated with microfracture and compared to the defect in the other knee treated with mosaic plasty. The age of the animals at surgery was 22 weeks. The follow-up was 12, 24 or 36 weeks following initial surgery.

The primary endpoint was difference in degree of tissue filling between the defects treated with microfracture and the defects treated with mosaic plasty at each follow-up. Secondary endpoints were differences in new bone formation within the defects, changes of the density of subchondral mineralized tissues and difference in degenerative changes evaluated by macroscopic appearance and joint fluid proteoglycan content between the defects treated with microfracture and those treated with mosaic plasty at each follow-up.

Animal care

The animal environment, diet, application of sterile perioperative conditions, capturing of synovial fluid for proteoglycan analyses, anesthesia, analgesia and the procedure of killing have been described previously [2]. Throughout the follow-up period, the experimental animals showed normal weight gain (Fig. 1).

The animals were allowed to move freely in their cages, and all animals were able to bear weight on both extremities immediately after surgery.

Surgery was performed by experienced orthopaedic surgeons certified according to the rules of animal care and experimental surgery. The study was carried out according to the guidelines for animal research at the University of Oslo and approved by the Norwegian Government Committee for Experimental Animal Care.

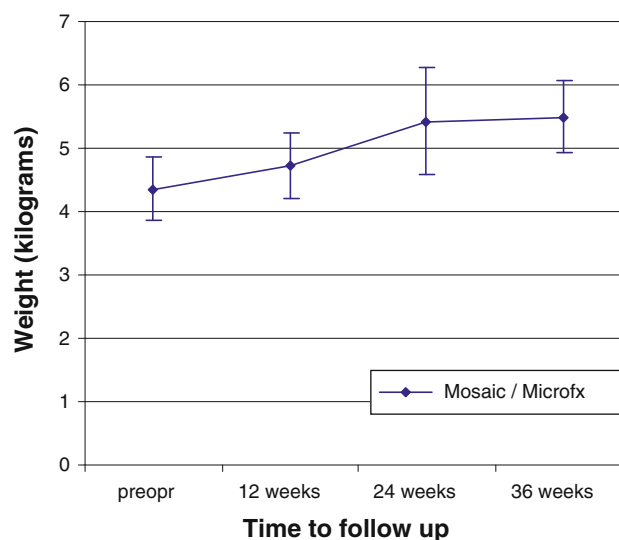


Fig. 1 The experimental animals gained weight throughout the experimental period. Mean weight at time zero was 4.35 kg. The number of animals at time zero (age 22 weeks) equalized the total number of animals evaluated at the follow-ups (37)—since they were all measured preoperatively. The number of experimental animals at 12, 24 and 36 weeks were 11, 10 and 16, respectively

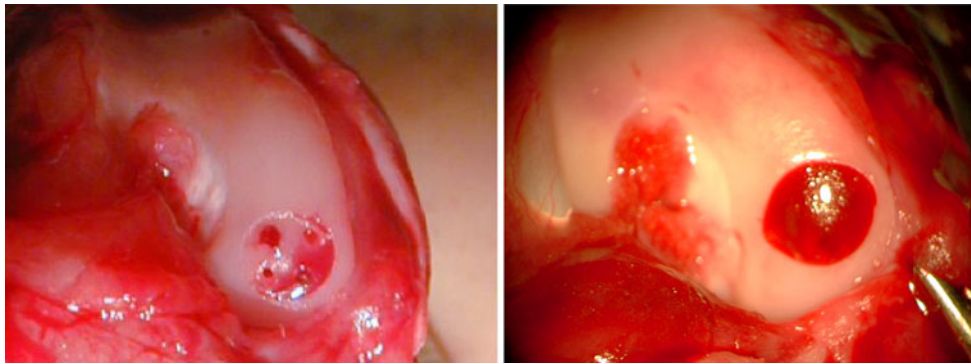


Fig. 2 Defect in MFC with the microfracture technique performed

Experimental groups

Forty adult New Zealand rabbits as planned and additionally four to compensate for early loss to follow-up were included as experimental animals. They all had defects created in both knees at age 22 weeks—in one knee treated with microfracture by randomization, whereas in the other knee treated with mosaic plasty. To avoid “learning curve” as a bias, the animals were block randomized for killing at the follow-up time points. Twelve animals were supposed to be killed 12 weeks postsurgery, 12 at 24 weeks and 16 at 36 weeks. Due to loss of animals during follow-up and other complications throughout the study, the final numbers of experimental knees treated with microfracture/mosaic plasty available for histological examination were 7/8 at 12-week, 8/10 at 24-week and 13/13 at 36-week follow-up. The numbers of animals available for paired analyses (sections from both knees available for evaluation) were 7 at 12 weeks, 8 at 24 weeks and 11 at 36 weeks.

Among the 44 animals (88 knees) undergoing cartilage repair, a washout sample of the synovial fluid containing a minimum of 0.75 ml collected prior to surgery (time zero) was available for proteoglycan content analyses from 86 knees. Due to loss of animals and exclusion of knees due to either patella dislocation or infection, the number of knees with samples both from time zero and at killing was reduced to 70. A total of 26 animals (52 knees) had samples available for analyses from both knees at both time zero and at killing.

Surgical technique

Through a medial parapatellar incision, a defect ($\varphi = 4$ mm) was created in the MFC of both knees as previously described for patella [2]. A stereomicroscope and small instruments were used, and care was taken to avoid any damage to the rims of the defects or to the underlying mineralized tissues. By randomization, the defect in one of the knees was treated with microfracture

using a specially designed awl and a mallet, the awl being tapped through the subchondral cortical bone plate establishing four equally spaced penetrating channels to the blood-filled lacunas of the subchondral miscellaneous bone (Fig. 2). The diameter of the channels was proximally 0.8 mm. The defect of the other knee was treated with mosaic plasty, using a 1.4-mm cylindrical motorized chisel harvesting osteochondral plugs close to the intercondylar notch. In the defect, three recipient sockets were made using a regular 1.4-mm burr. The grafts were transferred to the sockets and left with press fit fixation flush to the cartilage surrounding the defect (Fig. 3). Due to the fragility and consequently breakage of some grafts, sometimes four and occasionally five grafts were harvested. The joint was irrigated, hemostasis applied and wound closure performed as previously described [2].

Killing of animals, macroscopic evaluation and preparations for histological analyses

The animals were killed and synovial fluid was obtained as previously described [2]. The femoral condyles were



Fig. 3 Defect in MFC with the mosaic plasty performed

dissected free and gross morphologic grading was performed. Changes to the cartilage corresponding to ICRS grades 1–2 and/or small osteophytes were characterized as minor changes, whereas changes exceeding that were characterized as major. The specimens were fixed by immersion in phosphate-buffered 4% paraformaldehyde for 1 week and decalcified in 20% formic acid until the bone was soft enough for sectioning. Cubes, measuring 8 by 8 mm containing the defect at one side, were harvested from the specimens, dehydrated in graded alcohol and embedded in an epoxy resin (Agar 100[®], Agar Scientific, Stanstead, UK).

Histology

The cubes were sectioned from one longitudinal surface, the anterior–posterior and medial–lateral orientation of the defect at random [25]. From the point where the rim of the defect was reached, sections—each 1–2 μm thick—were captured at five different levels, each level 700 μm further into the defect. Sections from the three most central of the five levels were used for evaluation. This technique ensured that those three sections would all be within a maximum distance of 1050 μm from the very center of the defect. The sections were stained with toluidine blue and micrographed at 40 \times magnification using a digital camera mounted on the microscope (Color View III[®], Olympus Soft Imaging Solutions, Münster, Germany). An interactive semiautomatic image analysis program (Analysis Pro[®], Olympus Soft Imaging Solutions, Münster, Germany) was used for all measurements and the results were expressed in μm —i.e., mm with three decimals. The mean values of the three sections closest to the center of each defect (selected by the largest diameters) were included in the statistical analyses. The observer was blinded to the technique used for treatment for the defects.

Estimation of tissue filling

The borders of the defects were identified by the interfaces between presumed original cartilage and newly formed fibrocartilaginous/fibrous repair tissue on both sides. The tidemark was identified. If the tidemark was partially disrupted, the level of tidemark between identifiable fractions was estimated taking the curvature of the condyle into account. The midpoint along the tidemark of the defect was defined. From the midpoint, sectors of 500 μm length were marked along the tidemark to each side until 2 mm from midpoint was reached on each side, representing the tidemark at the base of the “shoulders” of the 4-mm defect. Cartilage height was measured at these two shoulder points, as was the height of new bone formation above the level of the tidemark and total tissue height at the seven

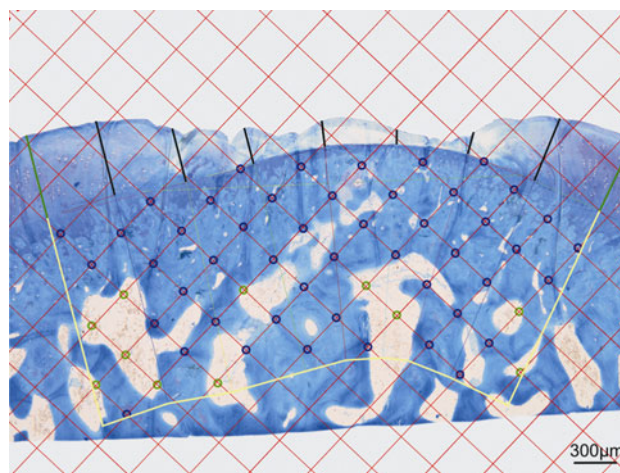


Fig. 4 Histological section of a defect stained with toluidine blue and micrographed at 40 \times magnification. The section is modified highlighting some of the graphics superimposed by the software Analysis Pro[®]. The two green lines outline the height of the shoulders measured. The seven black lines indicate the height of the tissue filling the defect. The yellow lines together with the tidemark frame the area of interest for subchondral mineralized tissue evaluation. The intersections of the red grid are marked with blue circles whenever overlying mineralized tissue and green circles whenever not

intersection points of the 500- μm -length sectors (Fig. 4). New bone formation and total tissue filling were estimated by relating the mean height of tissue at the seven defined points to the mean cartilage height of the two shoulders of the same defect, expressed as percentage filling with one decimal. Whenever one of the shoulders was technically not measurable, the one shoulder left served as the reference. This occurred in 15 sections from nine knees treated with microfracture and in nine sections from six knees treated with mosaic plasty.

Estimation of the density of subchondral mineralized tissue

The density of the subchondral mineralized tissue was estimated in an area immediately 1500 μm deep to the tidemark of the defect, the shape of the region of interest depending on the curvature of the tidemark (Fig. 4). Morphometry was performed by point counting [25] using computer software. A grid with 300 μm between test lines was superimposed on the micrograph. The intersections between the test lines served as test points. Subchondral mineralized tissue density was expressed as the number of test points overlying mineralized tissue relative to the total number of test points within the region of interest. The counting was repeated twice for each section with the orientations of the grid randomly selected at both occasions. The mean value of the two measurements was used for further statistical analysis, and the results were given with one decimal. A similar technique was used by Løken

et al. [46] demonstrating a variance less than 10% between measurements and between observers.

Analysis of synovial fluid

Proteoglycan content analyses were performed using standard ELISA technique [54]. The samples were all analyzed as one batch. Only knees with samples from both time zero and at killing were included in the statistical analyses. The value of proteoglycan content at time zero was subtracted from the value at killing, and the mean change, named delta (Δ), was compared between groups.

Statistical analysis

Based on previous experimental studies [1], a filling difference of more than 25% was considered being a proper level to discard the H_{01} hypothesis of no difference in tissue filling between the knees treated with microfracture and those treated with mosaic plasty. Since the experimental animals all underwent cartilage repair in both knees, with microfracture in one and mosaic plasty in the other, they would served as their own control regarding tissue filling of the defects, thus a paired Student *t*-test could be applied. Pre-experimental analysis to detect sample size using a power of 0.80 and a significance level of 0.05 and a standard deviation for the differences of less than 24% indicated a need of nine animals in each group for this purpose. To evaluate the difference in tissue filling from one follow-up time point to another regarding each treatment separately, the need of animals was estimated to 12 due to an unpaired experimental situation. Power and sample size estimations were not performed for the secondary endpoints. To cover up for loss during follow-up, we initially included 40 rabbits in the study. However, due to early loss of animals during surgery and follow-up, the experimental group was expanded to 44 rabbits.

The differences in tissue filling, new bone formation and subchondral mineralized tissue density between defects treated with microfracture and those treated with mosaic plasty—and the changes with time—were evaluated by one-way ANOVA, post hoc Bonferroni and paired Student *t*-tests. The change in synovial fluid proteoglycan content from time zero to follow-up, named delta (Δ), was evaluated by one-way ANOVA, post hoc Bonferroni and paired Student *t*-tests as well. Interactions between time and treatment technique were investigated; dependent observations on the individual level were accounted for by computing pairwise the difference between the deltas of the knees, the results applied in an one-way ANOVA model with time to follow-up as group factor. The rise in proteoglycan content (delta) for each location at each follow-up was further analyzed by paired Student *t*-tests and the

level of significance corrected according to Bonferroni. Results are given as mean and standard deviations (SD). SPSS statistical package version 14 (Chicago, Illinois, USA, 2005) was used for statistical analysis.

Results

Macroscopic changes

Among the knees with cartilage repair but no complications, none had major degenerative changes at any follow-up. Some minor degenerative changes were observed in twelve of the 70 treated knees available for evaluation (Table 1). None of the variables new bone formation (ns), subchondral mineralized tissue density (ns) or change in synovial fluid proteoglycan content (ns) were correlated with the minor degenerative changes observed.

Tissue filling of the chondral defects

Overall, mosaic plasty resulted in a significant higher degree of total filling of the defects than did microfracture ($P = 0.010$) (Table 2). There was no significant effect of time on the difference between mosaic plasty and microfracture. However, from being similar at 12 weeks, the difference in filling increased to significantly 34% at 36-week follow-up (Fig. 5).

New bone formation within the chondral defects

Overall, there was significantly more new bone formation within the defects treated with mosaic plasty compared to microfracture ($P = 0.010$) (Table 3). There was no significant effect of time, neither for mosaic plasty and microfracture separately, nor for the difference between them.

Subchondral mineralized tissue density

Overall, the density of mosaic plasty treated condyles was significantly lower than that of microfracture-treated

Table 1 The fraction of knees with minor degenerative changes observed macroscopically at follow-up

	12 weeks	24 weeks	36 weeks
Knees treated with microfracture technique	1/10	2/10	1/15
Knees treated with mosaic plasty	1/11	3/10	4/14

There was no difference in the frequencies of changes between microfracture and mosaic plasty, and there was no effect of time to follow-up

Table 2 Total amount of tissue filling in defects treated with mosaic plasty and microfracture, pairwise compared at the different follow-up time points

	12 weeks	24 weeks	36 weeks
Mosaic plasty	31.8% SD 17.6 95% CI [15.5–48.1] (n = 7)	45.5% SD 30.3 95% CI [20.1–70.8] (n = 8)	65.5% SD 38.0 95% CI [39.9–91.0] (n = 11)
Microfracture technique	31.6% SD 17.7 95% CI [15.2–47.9] (n = 7)	30.2% SD 17.4 95% CI [15.7–44.7] (n = 8)	31.4% SD 13.4 95% CI [22.4–40.4] (n = 11)
Mean paired difference Mosaic-Microfx	0.2% SD 22.5 95% CI [–20.5 to 21.0]	15.3% SD 40.2 95% CI [–18.4 to 48.9]	34.0% SD 33.9 95% CI [11.3–56.8]
<i>P</i> value	(ns)	(ns)	0.008

ns, Non-significant

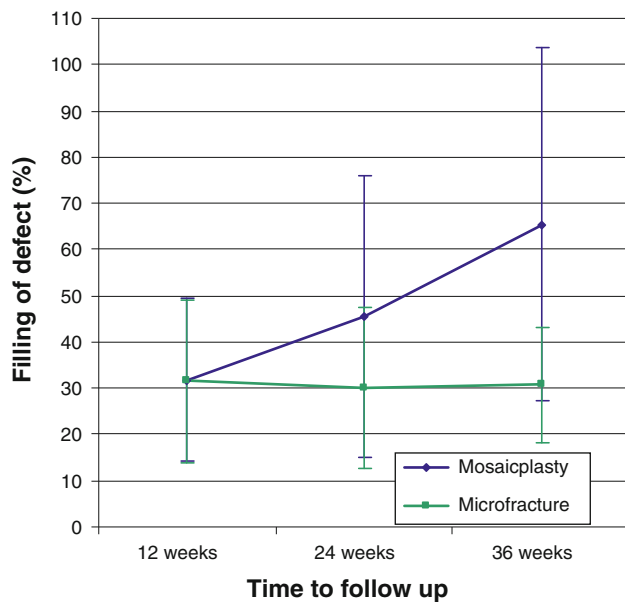


Fig. 5 Percentage filling of the defects treated with mosaic plasty and microfracture technique at the different time points of follow-up. For pairwise comparison, the number of animals at 12-, 24- and 36-week follow-up was 7, 8 and 11, respectively

condyles ($P = 0.001$) (Table 4). Although there was no significant effect of time, the difference between microfracture and mosaic plasty appeared significant only at 36-week follow-up (Fig. 6).

Synovial fluid proteoglycan content

There was a significant rise in synovial fluid proteoglycan content from the time of surgery to follow-up in the knees treated with microfracture ($P = 0.004$) as well as in those treated with mosaic plasty ($P < 0.001$), with no significant effect of time to follow-up (Table 5). There was an overall tendency of a higher rise of proteoglycan content in knees

treated with mosaic plasty than in those treated with microfracture, the difference, however, being significant at 12-week follow-up only (Fig. 7).

Complications

No animals died prior to surgery. Two of the animals died during surgery, the reason being unknown. They were both replaced. Among the others, visual observations did not detect any harmful effects on gait postoperatively, and no difference in level of activity or motion pattern between the two legs was observed. Six of the animals sustained sudden unexpected death during follow-up, the reason being unknown. Two of these animals died within 3 weeks after surgery and were replaced. The other four deaths were within 2 weeks from a follow-up time point, and except one knee with patellar dislocation, the knees were all unaffected and the specimens therefore maintained in the study. Three animals had to be killed during follow-up due to impaired general health conditions. In two of these, the knees had to be excluded, whereas in one rabbit, both knees were unaffected, the time of killing was within 2 weeks from a follow-up time point, and the specimens therefore maintained in the study. Among the total population of animals, complications related to the knee were observed in 24% of the knees: Three knees (3.4%) were excluded due to patellar dislocation, six knees (6.8%) because of infection and twelve knees (13.6%) due to technical failures in histology preparation.

Discussion

The major finding of the current experimental study was that neither of the two evaluated techniques for cartilage repair resulted in altogether good and predictable outcome regarding the parameters observed in this long-term

Table 3 New bone formation within defects treated with mosaic plasty and microfracture, pairwise compared at the different follow-up time points

	12 weeks	24 weeks	36 weeks
Mosaic plasty	2.0%	13.3%	12.0%
	SD 3.0	SD 12.8	SD 10.6
	95% CI [-0.8 to 4.7]	95% CI [2.5–24.0]	95% CI [4.9–19.1]
	(<i>n</i> = 7)	(<i>n</i> = 8)	(<i>n</i> = 11)
Microfracture technique	4.9%	1.4%	2.5%
	SD 7.4	SD 2.6	SD 3.3
	95% CI [-1.9 to 11.7]	95% CI [-0.7 to 3.6]	95% CI [0.3–4.7]
	(<i>n</i> = 7)	(<i>n</i> = 8)	(<i>n</i> = 11)
Mean paired difference Mosaic-Microfx	-2.9%	11.8%	9.5%
	SD 9.2	SD 14.0	SD 10.9
	95% CI [-11.4 to 5.6]	95% CI [0.1–23.5]	95% CI [2.2–16.8]
<i>P</i> value	(ns)	0.048	0.016

ns, Non-significant

Table 4 Subchondral mineralized tissue density immediately deep to the chondral defects treated with mosaic plasty and microfracture, pairwise compared at the different follow-up time points

	12 weeks	24 weeks	36 weeks
Mosaic plasty	68.6%	69.4%	63.9%
	SD 6.9	SD 3.4	SD 7.3
	95% CI [62.3–75.0]	95% CI [66.5–72.3]	95% CI [58.9–68.8]
	(<i>n</i> = 7)	(<i>n</i> = 8)	(<i>n</i> = 11)
Microfracture technique	74.4%	70.1%	70.4%
	SD 8.6	SD 5.1	SD 7.9
	95% CI [66.5–82.4]	95% CI [65.8–74.4]	95% CI [65.4–74.5]
	(<i>n</i> = 7)	(<i>n</i> = 8)	(<i>n</i> = 11)
Mean paired difference Mosaic-Microfx	-5.8%	-0.7%	-6.5%
	SD 6.9	SD 2.4	SD 7.2
	95% CI [-12.2 to 0.6]	95% CI [-2.7 to 1.3]	95% CI [-11.3 to -1.7]
<i>P</i> value	(ns)	(ns)	0.013

ns, Non-significant

follow-up. This is in line with some of the clinical publications from randomized studies using the same techniques [6, 38].

Effect of microfracture and mosaic plasty on tissue filling of the defect

The present study demonstrated an overall higher degree of total tissue filling in the defects treated with mosaic plasty compared to those treated with microfracture. The difference was 0.2% at 12-week, 15.3% at 24-week and significantly 34% at 36-week follow-up, the filling of microfracture defects remaining unchanged. We did not, however, have enough power in the study to detect this increase as a significant effect of time, neither separately for mosaic plasty filling, nor for the computed difference between mosaic plasty and microfracture.

A large variance in the degree of filling was noted with both treatment modalities. Such a large variance in the degree of filling of pure chondral defects has previously

been brought to attention regarding both untreated defects [8, 28] as well as defects treated with microfracture [7, 41]. The variability in filling of the interstitial space between the grafts in mosaic plasty has been underlined as well [35, 36].

Tissue filling is considered to be an important variable in restoration of cartilage defects. However, the critical amount of tissue filling necessary to significantly discriminate one clinical outcome from the other concerning joint function, pain, disability and reduced risk of osteoarthritis is not well understood. Moreover, the correlation between tissue filling and other histological findings on one hand, and functional outcome on the other, is not obvious [17, 39, 47]. According to our power and sample size estimation, a filling difference of more than 25% was considered as a proper level to discard the H_{01} hypothesis of no difference in tissue filling between treatment modalities. Thus, the 34% higher degree of filling following mosaic plasty compared to microfracture at the latest follow-up discarded our H_{01} hypothesis.

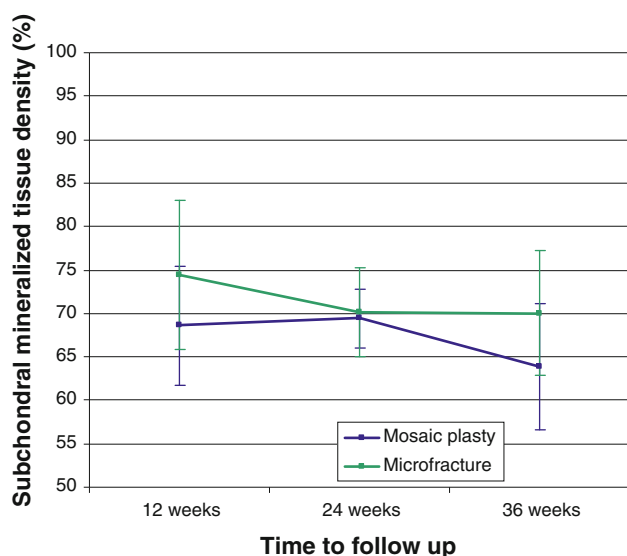


Fig. 6 Subchondral mineralized tissue density immediately deep to the defects treated with mosaic plasty and microfracture technique at the different time points of follow-up. For pairwise comparison, the number of animals at 12-, 24- and 36-week follow-up was 7, 8 and 11, respectively

Although not part of the same study, a natural history study from our group [28] revealed significantly less filling in 35 identical control defects when compared to the present surgical repair, both when compared to microfracture (less than 25% difference at all time points) as well as when compared to mosaic plasty (45% difference at 36 weeks).

There are to our knowledge only two clinical and no experimental studies comparing these two techniques for cartilage repair. In their clinical studies, Gudas et al. [24] demonstrated superior results of mosaic plasty compared to microfracture clinically, macroscopically, histologically and radiologically 37 months following treatment. Tissue filling of the defects was, however, not evaluated.

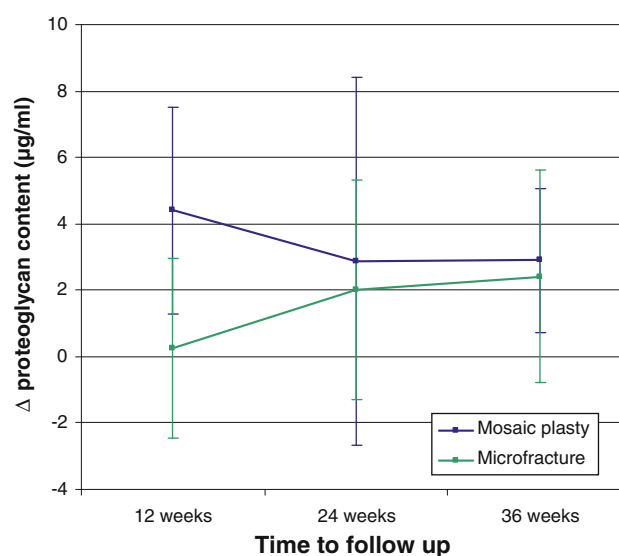


Fig. 7 Change (Δ) in synovial fluid proteoglycan content ($\mu\text{g/ml}$) from time zero to the different time points of follow-up in knees treated with mosaic plasty and microfracture technique. For pairwise comparison, the number of animals at 12-, 24- and 36-week follow-up was 10, 10 and 14, respectively

In animal models on the other hand, tissue filling has been studied following microfracture [7, 16, 19, 20, 31]. Although the majority of studies on microfracture report tissue filling superior to the 31.4% observed at 36 weeks in the present study, only a limited number of papers present an increase in filling of more than 25% compared to controls [16]. This is in agreement with our study. The reason we did not obtain the same degree of tissue filling as reported in some other studies may have several explanations, such as the anatomical and biomechanical differences between species [13], the intraarticular location [7, 9, 20, 28, 40], the depth of the lesion [17, 34], the size of the defect [44] and the technique of which the penetrations to the bone marrow was performed [15]. In the current study,

Table 5 Rise (Δ) in synovial fluid proteoglycan content ($\mu\text{g/ml}$) from time zero to follow-up in knees treated with mosaic plasty and microfracture, pairwise compared at the different follow-up time points

	12 weeks	24 weeks	36 weeks
Mosaic plasty (Δ)	4.4	2.9	2.9
	SD 3.1	SD 5.5	SD 2.2
	95% CI [2.1–6.5]	95% CI [–1.1 to 6.8]	95% CI [1.7–4.1]
	(<i>n</i> = 10)	(<i>n</i> = 10)	(<i>n</i> = 14)
Microfracture technique (Δ)	0.2	2.0	2.4
	SD 2.7	SD 3.3	SD 3.2
	95% CI [–1.7 to 2.2]	95% CI [–0.4 to 4.3]	95% CI [0.7–4.1]
	(<i>n</i> = 10)	(<i>n</i> = 10)	(<i>n</i> = 14)
Mean paired difference Mosaic-Microfx	4.2	0.9	0.5
	SD 4.6	SD 5.9	SD 4.1
	95% CI [0.9–7.5]	95% CI [–3.3 to 5.1]	95% CI [–1.8 to 2.9]
<i>P</i> value	0.018	(ns)	(ns)

ns, Non-significant

bleeding from the microfracture channels varied, both from one pick hole to another within a defect and from one animal to another. This phenomenon has been described by other authors as well [7].

Due to large disparity between studies, experimental comparisons of different treatment modalities and techniques should be performed in the same animal model. Although rabbits are the most widely used animal model for cartilage restoration in general [4, 20], this is the first study applying the MFC of the rabbit knee as a model for mosaic plasty.

At 36-week follow-up, the filling of the mosaic plasty defects was 65.5%, more than twice that of microfracture and significantly 45% higher than the 20.5% previously observed in untreated defects [28]. In previous literature concerning mosaic plasty, the evaluation of tissue filling has not been a focus of attention. Mosaic plasty implies the transplantation of several circular osteochondral autografts to a chondral defect where the grafts are implanted in precisely drilled recipient holes to resurface the defect by press fit graft to wall friction—composing a “mosaic”. Accuracy of graft delivery such as perpendicularity of the grafts, adequate press fit fixation to insure sufficient stability, and the grafts not being left prone to the surrounding surface is associated with success—avoiding graft subsidence and overgrowth with fibrous tissue [27, 35, 58]. The use of circular grafts initially leaves some denuded interstitial space in between the cartilage of the grafts and toward the surrounding cartilage. These gaps are supposed to heal by “cartilage flow” and extrinsic repair [35]. The gradual filling of the interstitial spaces may contribute to an increased amount of tissue filling with time—not in conflict with the current study. In the present study, we used three 1.4-mm grafts in a 4-mm defect, covering 37% of the defect—thus corresponding to an initial 37% tissue filling—if the delivery was appropriate. The mean tissue filling of the defects was 31.8% at 12-week, compared to 65.5% at 36-week follow-up. The lower value of tissue filling at 12 weeks compared to the theoretically initial filling might be due to subsidence of grafts and lack of filling of the interstices in some defects. The 65.5% filling achieved at 36 weeks is likely due to filling of the interstices and/or overgrowth of the grafts with fibrous tissue.

There was a great variance in the results following mosaic plasty at 36 weeks (SD 38.0%, 95% CI [39.9–91.0]). Since tissue filling exceeding 37% was dependent on cartilage flow and extrinsic repair, our findings are in agreement with previous studies suggesting that this kind of repair is unpredictable [35, 36].

Quantitative measures of tissue filling following mosaic plasty add valuable information on the overall consequences of both graft subsidence and interstitial filling. Moreover, evaluating defect filling by measuring the height

of tissue above the level of the tidemark—as in the present study—reflects one of the main proposed advantages of mosaic plasty, namely preserving the joint surface congruity. To our knowledge, the present study is the first to evaluate mosaic plasty by quantitative measure of tissue filling, making it possible to compare the results to other cartilage repair techniques such as microfracture.

Effect of microfracture and mosaic plasty on new bone formation within the defects

The current study demonstrated that both microfracture and mosaic plasty resulted in new bone formation above the level of the tidemark. New bone formation following mosaic plasty comprised considerable 13.2 and 12.0% of the defect volume at 24 and 36 weeks, respectively, significantly different from the 1.4 and 2.5% following microfracture. By comparison, untreated defects previously reported showed no signs of new bone formation above tidemark at any follow-up [28]. New bone formation in defects treated with microfracture has been reported previously [16–19] as well as in defects treated with mosaic plasty [33]. The implications of new bone formation observed in cartilage repair are, however, not known. On the other hand, new bone formation reflected as tidemark—and thereby calcified tissue—advancement into the non-calcified cartilage has been associated with the progression of OA [14] and therefore is a matter of concern—also in regards to cartilage repair.

Effect of microfracture and mosaic plasty on subchondral mineralized tissue density

The current study showed an overall significant lower subchondral mineralized tissue density in mosaic plasty-treated defects compared to those treated with microfracture, the significance being apparent at the latest time point of follow-up. To our knowledge, this is the first study comparing the effect of these two cartilage repair techniques on subchondral bone. Compared to untreated defects reported previously [28], mosaic plasty resulted in a significant reduction in the subchondral mineralized tissue density ($P = 0.002$), whereas microfracture did not (ns). Alterations in the subchondral bone following mosaic plasty or separate/single osteochondral autograft transfer have been reported by other authors as well [26, 35, 43, 58, 63, 68]. The natural history bony reaction is described as early bone resorption followed by remodeling—resembling fracture healing. This remodeling process seems to be complete at 12 weeks in rabbits [55] and at 6 months in larger animals [35, 42, 43, 63]. Persistent subchondral cyst-like lesions or cavities have been described in association with proud grafts [58], angled grafts/graft subsidence or

graft instability [35, 68], and with too early weight bearing [26]. In the current study, the grafts were introduced perpendicular to the articular surface, flush with the surrounding cartilage, and with assumingly good press fit fixation. Subchondral bone changes are associated with the initiation and progression of OA [14, 61]. However, no consensus has been reached regarding the pattern of changes or regarding the degree of changes at which initiation and/or progression of OA could be anticipated. Whether the statistically significant changes we did observe in the present study do have any clinical implications thus remain uncertain, however, they still are a matter of concern. Although grading of subchondral bone changes has been included in different scoring systems for cartilage restoration [50, 51, 56, 59, 60, 69], the scores are semi quantitative. To our knowledge, quantitative analyses of the subchondral mineralized tissue density following cartilage repair have not previously been thoroughly investigated.

Synovial fluid proteoglycan content

Mosaic plasty resulted in a significantly higher rise of synovial fluid proteoglycan content compared to microfracture at 12-weeks follow-up. There was no difference at 24- and 36-week follow-up. Increased concentrations of proteoglycan fragments in the joint fluid have been associated with trauma and surgery in humans [45, 57] and correlated with increasing OA in a rabbit model [53]. In their rabbit model, Messner and coworkers demonstrated values being elevated at 3 months, decreasing to normal values at 6 months and then increasing again at 12 months. The rise at 3 months was explained as possibly surgery related in line with clinical findings [57], whereas the rise at 12 months was explained as possibly related to initial degeneration. Their observations are not in conflict with the current study. The significant difference in rise seen at 12 weeks may reflect that mosaic plasty is more traumatizing to the joint than is microfracture. On the other hand, synovial fluid analyses did not indicate a degenerative process in neither of the groups during the observation period.

Limitations, strengths and clinical relevance of the current study

One limitation of the current study was the insufficient number of animals which left us with a power problem in evaluating the effect of time. The material suffered from a high number of complications and failures in histological preparation. Two animals died during surgery, and six animals died unexpected in the follow-up. However, the data obtained at 36 weeks represent the most valuable

information from the current study. Complications and loss of animals related to the rabbit model have previously been described by our group [28] as well as by other authors [10].

The importance of defining the depth of experimentally produced defects in relation to the different layers of the joint organ—and evaluating the results in relation to that—has been emphasized [8, 10, 14, 34]. Even the bias of not removing all the tissue of a layer as intended, or causing damage to the layer beneath, has been a topic in discussing the results of cartilage restoration [8, 17, 19]. Methodologically, this study was strengthened by the use of a stereomicroscope during surgery. Without that, the procedure demands great care and is hard to perform without leaving residual cartilage or damage to the subchondral mineralized tissues [7, 8, 31]. Additionally, a commonly applied experimental model was used to systematically investigate discrepancies in two techniques widely used for cartilage repair. The study was randomized and controlled, and the histological sections for analyses were blinded to the investigator. The main outcome measures were all quantitative.

Clinically, cartilage repair techniques possess three goals: (1) complete filling of the defect, (2) restoration of normal cartilage morphology and (3) restoration/preservation of the subchondral mineralized tissues. This study illustrated possible shortcomings in two of our current techniques in cartilage repair. Although there are statistically significant differences in tissue filling and subchondral mineralized tissue changes, the study demonstrated that mosaic plasty and microfracture both do have unpredictable results in the single case—even in a standardized experimental model. This unpredictability is not unfamiliar in clinical case series [64, 65] and is of concern for both techniques in order to re-establish the anatomy of knee joint cartilage.

Conclusion

Mosaic plasty resulted in a higher degree of tissue filling but affected the subchondral mineralized tissues more than microfracture. Insufficient filling occurred following both techniques. The study demonstrated experimentally that mosaic plasty and microfracture both do have unpredictable results in the single case—even in a standardized animal model. The study underlines that experimental studies evaluating cartilage repair necessitate long-term follow-up to reveal relevant results.

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