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Sustained superiority in KOOS subscores after matrix-associated chondrocyte implantation using spheroids compared to microfracture

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Abstract

Purpose To evaluate the safety and efficacy of matrix-associated autologous chondrocyte implantation (ACI) using spheroids in comparison to arthroscopic microfracture for the treatment of symptomatic cartilage defects of the knee.

Methods In a prospective multicenter-controlled trial, patients aged between 18 and 50 years, with single symptomatic focal cartilage defects between 1 and 4 cm^2 (mean 2.6 ± 0.8, median 2.75, range 1.44–5.00) in the knee were randomized to treatment with ACI with spheroids ($n = 52$) or microfracture ($n = 50$). Primary clinical outcome was assessed by the Knee Injury and Osteoarthritis Outcome Score (KOOS). Analyses were performed in a defined hierarchical manner where outcomes of ACI were first compared to baseline values followed by a comparison to the microfracture group with repeated-measures ANCOVA with a non-inferiority approach. Subgroup analyses were performed to investigate the influence of age and defect size on the overall KOOS. Secondary clinical outcomes were the magnetic resonance observation of cartilage repair tissue (MOCART), modified Lysholm score and International Knee Documentation Committee (IKDC) examination form. Safety data focused on adverse events. Here the 5 years results are presented at which there were 33 observed cases in the ACI group and 30 in the microfracture group.

Results The overall KOOS and its five subscores were significantly improved compared to baseline for both the ACI and microfracture group. Non-inferiority of ACI to microfracture was confirmed for the overall KOOS and the subscores, while for the subscores activities of daily living, quality of life and sports and recreation of the threshold for superiority was passed. In the ACI group, a notably more rapid initial improvement of the KOOS was found at three months for the older age group compared to the younger age group and the microfracture group. No other differences were found based on age or defect size. In addition, clinical improvement was found for the MOCART, modified Lysholm and IKDC examination form both the ACI and microfracture group. No safety concern related to either treatment was observed.

Conclusion This study confirms the safety and efficacy of matrix-associated ACI with spheroids at a mid to long-term follow-up. Non-inferiority of ACI to microfracture was confirmed for the overall KOOS and all subscores, while superiority was reached for the subscores activities of daily living, quality of life and sports and recreation in the ACI group. This underlines the importance of ACI for the young and active patients.

Level of evidence. I.

Keywords Autologous chondrocyte implantation · Cartilage lesion · Cartilage defect · Knee surgery · MOCART · KOOS · Randomized clinical trial

Introduction

Cartilage repair therapies have gained increasing interest over the last decades as symptomatic cartilage lesions severely impair patients´ activity levels in sports as well as daily living [20, 21, 30]. Several treatment options have been introduced with significant improvement of joint function and pain reduction. Since the introduction of autologous

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chondrocyte implantation (ACI), this technique has evolved in several generations. Due to the increasing scientific evidence, ACI has been recommended by several national and international medical associations as first line treatment for medium to large sized cartilage defects [7, 8, 24]. These recommendations are reflected by several high level studies that have compared ACI with other treatment options like microfracture or osteochondral transplantation (OCT) [2, 3, 13, 16, 20, 34]. Superior composition and structural properties of the repair tissue compared to bone-marrow stimulating procedures is regarded as one of the key factors for more favorable outcomes after ACI [9, 35]. This likely contributes to the sustained clinical improvement after ACI, which has been reported up to 20 years [29], while a deterioration over time has been observed for microfracture [22]. However, longer follow-up periods have so far mostly been reported for first and second generations of ACI [3, 16, 29]. It is important to analyze the longer follow-up outcomes of more recent generations of ACI to determine improvements compared to the first generations ACI [23, 28, 32]. One of the more recent generations ACI is a matrix-associated ACI with spheroids. In this method the chondrocytes are culture expanded in a monolayer followed by 3D culture forming spheroids that consist of chondrocytes and their self-synthesized extracellular matrix. The spheroids are *delivered* in a NaCl solution and are self-adhesive to the subchondral bone and cartilage. This, in contrast to other ACI´s, eliminates the need of any exogenous matrix, glue, or sutures. In addition, the application can be performed arthroscopically or minimal invasive. Several studies have already shown encouraging results of ACI with spheroids with a follow-up between one and five years [11, 12, 14, 27, 36–38]. Furthermore, a prospective randomized trial against arthroscopic microfracture has been performed with the hypothesis that ACI with spheroids is non-inferior to microfracture for the treatment of relatively small cartilage defects (1–4 cm^2). The two and three years follow-up results have been reported previously [12, 22]. Here, the final assessments of the full study duration of five years of this study are described. This is the first report on a prospective randomized trial evaluating the safety and efficacy of ACI using spheroids compared to arthroscopic microfracture in symptomatic cartilage defects of the knee at this length of follow-up. In addition, subgroup analyses on the influence of age and defect size on overall KOOS are described.

Material and methods

Study design and surgical treatment have been described previously [26]. This trial was a prospective, randomized, multicenter, clinical study in Phase 3 (NCT01222559; EudraCT Nr. 2009-016, 466-82) comparing the efficacy and safety of treatment with (a) matrix-associated ACI with spheroids (Spherox (Manufacturer CO.DON AG, Teltow, Germany), the ACI products was formerly called co.don chondrosphere®), to (b) microfracture. The trial was not blinded due to the required procedures of different treatments, but the central reader of the MRIs and the pathologist who assessed the biopsies were blinded.

The protocol and informed consent forms were approved by the ethics committees responsible for the respective centers and the local/national regulatory authorities (lead approval number 2009-070F-MA in Germany and UR.DBL.BLE.475.0154.2014 in Poland). Patients with written informed consent were included, between December 2010 and December 2014, at eight German and three Polish orthopedic centers. Patients were randomized using a fixed block size (6) stratified prospectively by age into two classes (18–34 and 35–50 years). Patients in each class were allocated randomly on a 1:1 basis to group A (ACI) or B (microfracture). The randomization was implemented by telephone by the clinical research organization during surgery as the exact defect size could not be determined earlier (criterion for inclusion in this study: 1–4 cm^2). The main inclusion criteria were: patients (18–50 years of age) with an isolated, symptomatic full-thickness cartilage defect (ICRS grade 3 or 4) with a defect size of 1–4 cm^2 after debridement to healthy tissue and a maximum depth of 6 mm, a nearly intact chondral structure surrounding the defect as well as the corresponding joint area and the willingness to accept restrictions on analgesics (only paracetamol and/or topical nonsteroidal anti-inflammatory drugs allowed during trial and discontinuation of pain medication required 1 week before each visit) and to follow the strict rehabilitation protocol and follow-up program. Main exclusion criteria were: defects in both knees at the same time; radiological signs of osteoarthritis, any signs of knee instability; valgus or varus malalignment ($> 5°$ over the mechanical axis); clinically relevant second cartilage lesion on the same knee, more than 50% resection of the meniscus in the affected knee or an incomplete meniscal rim; rheumatoid arthritis, parainfectious or infectious arthritis, or a condition after these diseases; pregnancy and planned pregnancy; obesity (body mass index > 30 kg/m^2); previous treatment with ACI in the affected knee; microfracture performed less than one year before screening in the affected knee; meniscal implant in the affected knee; meniscal suture (in the affected knee) 3 months before baseline; mosaicplasty (osteoarticular implant system) in the affected knee; hyaluronic acid intra-articular injections in the affected knee within 3 months before baseline; specific osteoarthritis drugs (such as chondroitin sulfate, diacerein, N-glucosamine, piascledine, or capsaicin) in the 2 weeks before baseline; corticosteroid treatment by an intra-articular route within the month before baseline or systemic (all routes) corticosteroids within 2 weeks before baseline; chronic use

of anticoagulants; and current diagnosis of osteomyelitis, human.

immunodeficiency virus (1 or 2), and/or hepatitis C infection.

Patient population

The study population comprised 102 patients aged 37 ± 9 years and the treatment groups were well balanced regarding demography and disease background (Table 1). The defects were located on the femur, except for one patient who had an additional defect on the patella (violating an inclusion criterion and microfracture was not performed). The mean (SD) pre-debridement defect size was 2.2 (0.7) cm^2 for the ACI group and 2.0 (0.8) cm^2 for the microfracture group, and post-debridement 2.7 (0.8) cm^2 and 2.4 (0.8) cm^2, respectively. The allowed post-debridement size of 1–4 cm^2 was exceeded for one patient. In both treatment groups, just below one-half of the defects were ICRS grade 3 and the remaining were grade 4. For the ACI group, a mean (SD) of 25.3 (16.4) spheroids/cm^2 defect after debridement were implanted.

A detailed illustration of the grouping and flow of patients within the trial is shown in Fig. 1. The intention-to-treat population consisted of 48 patients in the ACI group and 49 in the microfracture group, and at 60 months follow-up there were 33 and 30 observed cases, respectively.

Table 1 Patients' demographic and baseline data

Treatment group:	ACI $N=52$	Microfracture $N=50$	All patients $N=102$
Sex			
Female	19	22	41
Male	33	28	61
Age [years]			
Mean ± SD	36 ± 10	37 ± 9	37 ± 9
BMI [kg/m^2]			
Mean ± SD	25.7 ± 3.3	25.8 ± 3.0	25.8 ± 3.1
Range	18.8–31.2	18.2–30.0	18.2–31.2
Defect location (primary)			
Femur	52	49	101
Femur and patella	–	1[b]	1
Defect size [cm^2] pre-debridement			
Mean ± SD	2.2 ± 0.7	2.0 ± 0.8	2.1 ± 0.8
Range	0.5–3.5	0.8–4.0	0.5–4.0
Defect size [cm^2] post-debridement			
Mean ± SD	2.7 ± 0.8	2.4 ± 0.8	2.6 ± 0.8
Range	1.4–5.0[a]	1.0–4.0	1.0–5.0
Defect grade [ICRS]			
ICRS Grade 3	17	20	37
ICRS Grade 4	31	29	60

[a]One patient exceeded the allowed post-debridement defect size of 1–4 cm^2

[b]Violation of inclusion criterion as there was an additional defect on the patella for one patient, microfracture was not performed

ACI autologous chondrocyte implantation with spheroids, *SD* standard deviation, *BMI* body mass index, *ICRS* international cartilage regeneration and joint preservation society

Surgical techniques and rehabilitation

Treatment by ACI required two surgical interventions: a biopsy to obtain cartilage that was used to isolate and grow chondrocytes in vitro, and subsequent implantation of the chondrocytes in spheroids. The number of spheroids implanted was in the manufacturer's normal recommended range of 10–70 per cm^2 defect [28, 29]. One spheroid is formed by the self-aggregation of approximately 200.000 culture expanded chondrocytes and the viability is over 95% [40]. For microfracture, a single intervention was performed.

Following surgery, all patients followed a standardized rehabilitation protocol appropriate for the surgical treatments as reported previously [26]. Briefly, partial weight bearing was recommended for six weeks, continuous passive motion was started from the day after surgery, increasing within the first six weeks. Physiotherapy was adjusted to individual joint status and complaints and return to high impact sports earliest recommended after 12 months.

Assessment criteria

The Knee Injury and Osteoarthritis Outcome Score (KOOS) was assessed as primary clinical outcome measure. The primary endpoint was the change in KOOS compared to baseline at 24 months. This report describes the evaluation of secondary efficacy and safety outcomes of the full study duration of 60 months follow-up, comprising of: overall KOOS and KOOS subscores at other timepoints than 24 months, magnetic resonance observation of cartilage repair tissue (MOCART) scoring, modified Lysholm (24-point scale) and the International Knee Documentation Committee (IKDC) examination form. Patients were assessed at baseline and then 6 weeks and 3, 6, 12, 18, 24, 36, 48 and 60 months after treatment, except for the MOCART; as the MOCART assesses repair tissue, the first assessment was performed at 3 months and no MRI´s were performed at 6 weeks and 6 months. Results are reported with accuracy of one decimal.

Adverse events, vital signs, electrocardiography findings, physical examination findings, concomitant pain medication and laboratory values were documented as safety outcomes.

At 24 months follow-up, a second look arthroscopy was performed in a subset of patients who consented to this

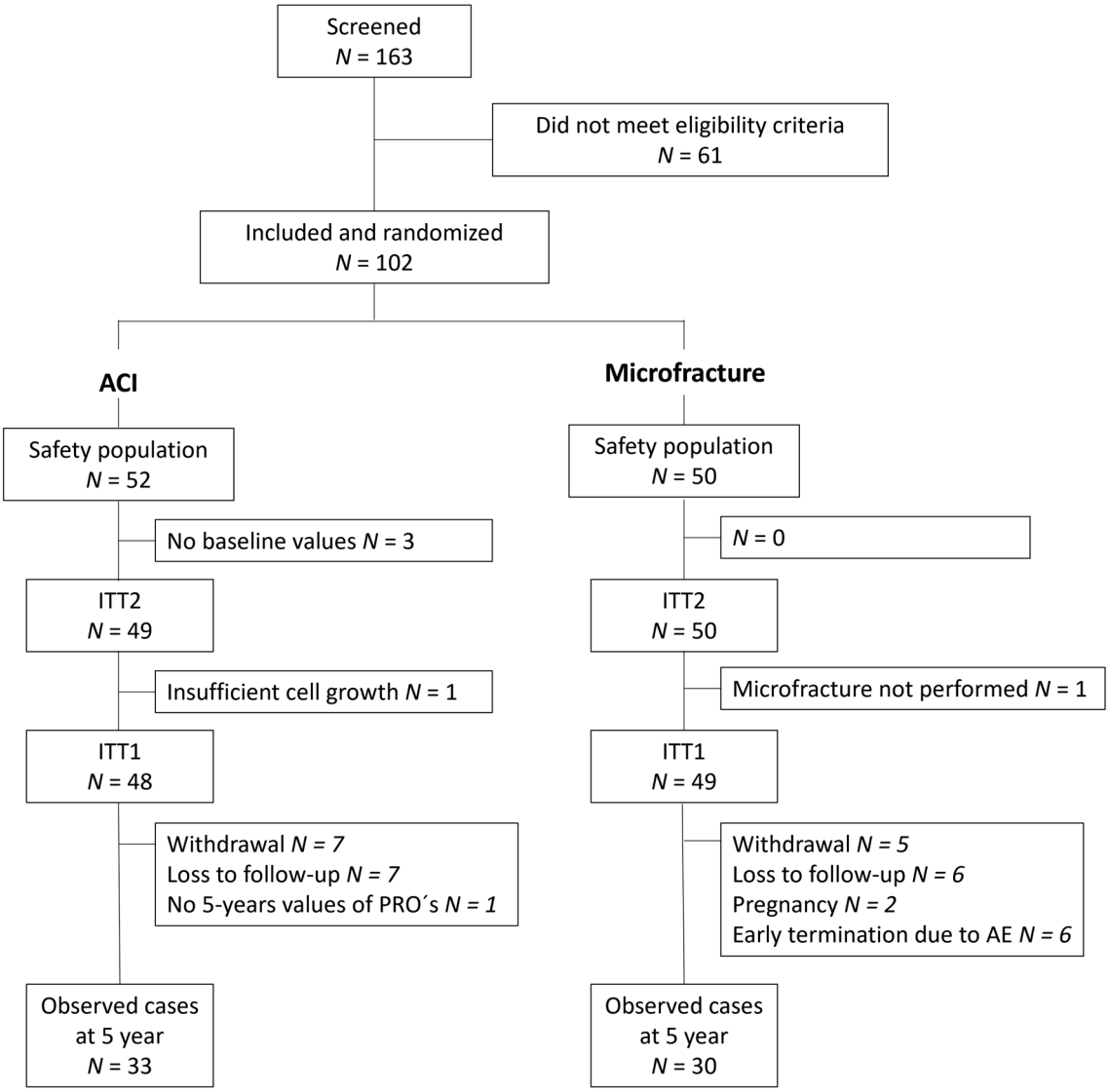

Fig. 1 Patient disposition in the clinical study. *ACI* autologous chondrocyte implantation, *ITT* intention to treat, *PRO* patient reported outcome, *AE* adverse event

additional procedure. The ICRS macroscopic repair score and histological findings have been previously reported [26].

Statistical analysis

The sample-size calculation considered the change from baseline in overall KOOS [33] and based on non-inferiority testing of ACI compared with microfracture (one-sided $\alpha = 0.025$, power = 80%, lower equivalence bound = − 8.5%, expected mean difference = 0, standard deviation = 15). A repeated-measure ANCOVA approach (including baseline, 6 weeks, 3, 6, 12 and 24 months) was used to estimate the overall effect and allowing for a sample-size reduction of 10% due to correlation. This resulted in a minimum sample size of $N = 90$ (i.e., 45 microfracture and 45 ACI). With an assumed drop-out rate of 7.5% for the microfracture group and 15.5% for the ACI group (greater for ACI than for microfracture because of the risk of an insufficient number of cells to perform the ACI treatment) the calculated sample size was 101 (49 microfracture and 52 ACI), but this was rounded up leading to a final sample size of $N = 102$ (50 microfracture and 52 ACI).

Non-inferiority analyses were performed with repeated-measures ANCOVA and abovementioned parameters. Clinical outcome was also assessed for each treatment group using a threshold of 8 percentage points as the minimal clinically important difference (MCID) from baseline in the overall KOOS [33]. Subgroup analyses stratified by age group and defect size. Differences between groups for modified Lysholm score and International Knee Documentation

Committee (IKDC) examination form were tested using ANOVA including baseline values. Differences between groups for IKDC grades were assessed using Kruskal–Wallis test. To test whether there was a correlation between overall KOOS and MOCART results, the Spearman´s correlation coefficient was calculated. The resulting *p*-values for all secondary variables are interpreted in an explorative sense. Each *p*-value ≤ 0.05 represents a significant result.

The intention-to-treat (ITT) population was defined as comprising all patients who (a) were randomized, (b) were treated by either ACI or microfracture, and (c) completed the KOOS questionnaire at baseline (Fig. 1). Single, individual missing scores after at least one post-treatment observation were imputed using Last Observation Carried Forward (LOCF) for the intent-to-treat assessment. The statistical analyses were performed by StatConsult GmbH (Magdeburg, Germany).

Results

Clinical outcome: primary assessment parameter KOOS

The overall KOOS score in the ACI group improved from a score of 56.6 ± 15.4 at baseline with 24.9 ± 17.4 points at the primary endpoint of 24 months and these scores were maintained throughout the 60 months follow-up (Fig. 2, Table 2). In the microfracture group, the overall KOOS increased from a score of 51.7 ± 16.5 at baseline with 21.5 ± 15.7 points at 24 months and were also maintained over the study duration (Fig. 2, Table 2). The improvement in overall KOOS was clinically relevant for the ACI group from 3 months onwards and for the microfracture group from 6 months onwards (Fig. 2).

The change from baseline in overall KOOS and all KOOS subscores at the primary end point of 24 months and at final follow-up of 60 months are shown in Table 2. For both the ACI and microfracture group, the change from baseline for the overall KOOS and all subscores was significant and the change from baseline for the overall KOOS exceeded the MCID at these timepoints. Non-inferiority of ACI to microfracture was shown for the changes in overall KOOS and it subscores at 24 and 60 months. Superiority on descriptive level of ACI over microfracture was observed for the KOOS subscore "activities of daily living" at 24- and 60 months follow-up, and for "quality of life" and "sports and recreation" at 60 months follow-up.

For the ACI group, 43 (90%) patients were considered responders with an increase in overall KOOS of at least 8 points at 60 months follow-up, for the microfracture group this was 41 (84%) patients.

Influence of age on the overall KOOS

The patients were stratified prospectively by age into two classes (18–34 and $\geq$ 35–50 years) by the randomization procedure. For the ACI-treated patients in the older group (18 patients), most improvement in change in overall KOOS compared to baseline was observed in the first 12 months with a clinically relevant improvement compared to baseline from 3 months onwards. From 18 months onwards there was still improvement in mean change in overall KOOS compared to baseline with a few points between each timepoint analyzed (Fig. 3A). Also in the ACI-treated patient in the younger group (30 patients), most improvement in change in overall KOOS compared to baseline was observed in the first 12 months, but clinically relevant improvement compared to baseline was observed from 12 months onwards. No other changes were observed in this group in the

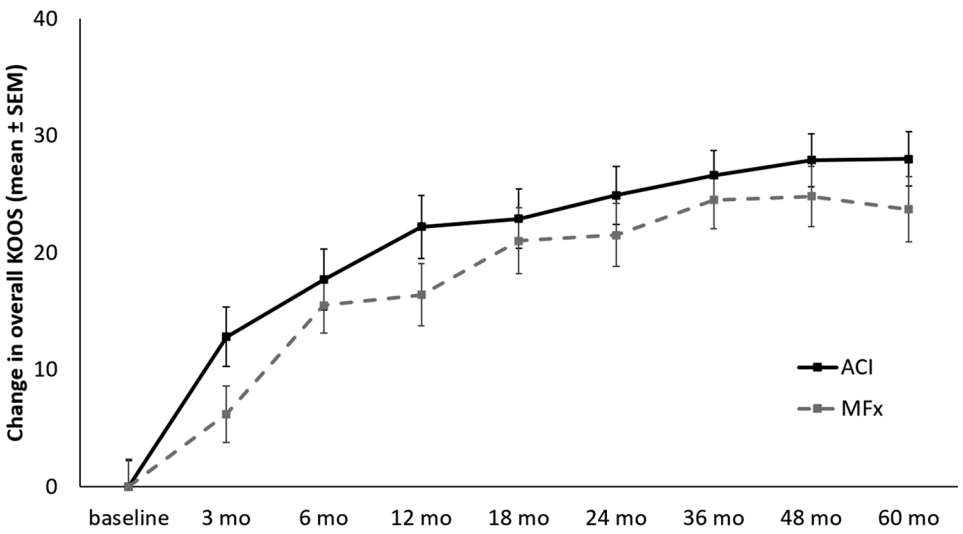

Fig. 2 Improvement in the overall Knee injury and osteoarthritis outcome score (KOOS) compared to baseline for the two treatment groups autologous chondrocyte implantation with spheroids (ACI) and microfracture (MFx)

Table 2 Baseline and change from baseline of overall KOOS and subscores at 24- and 60-months follow-up

	ACI *N=48*	Microfracture *N=49*	Difference	Lower 95% CI
Overall KOOS				
Baseline	56.6 ± 15.4	51.7 ± 16.5		
Change at 24 months	24.9 ± 17.4	21.5 ± 15.7	5.5	− 0.8
Change at 60 months	28.0 ± 18.4	23.7 ± 19.9	6.7	− 0.1
Responders at 60 months	43 (90%)	41 (84%)		
Pain				
Baseline	63.8 ± 18.5	58.0 ± 18.3		
Change at 24 months	22.5 ± 18.9	21.5 ± 16.3	4.2	− 2.0
Change at 60 months	23.4 ± 22.3	21.9 ± 20.4	4.7	− 2.6
Other symptoms				
Baseline	71.7 ± 16.0	66.8 ± 20.8		
Change at 24 months	15.8 ± 18.5	13.8 ± 15.1	5.0	− 0.4
Change at 60 months	17.4 ± 20.0	16.1 ± 21.3	4.3	− 2.0
Activities of daily living				
Baseline	71.5 ± 20.8	67.5 ± 20.0		
Change at 24 months	20.6 ± 17.2	16.6 ± 15.5	6.4	1.3
Change at 60 months	21.5 ± 18.3	18.0 ± 18.5	5.9	0.3
Sports and recreation				
Baseline	43.6 ± 25.7	36.1 ± 25.3		
Change at 24 months	30.7 ± 30.5	27.0 ± 25.0	8.1	− 1.9
Change at 60 months	37.2 ± 31.4	30.1 ± 28.6	11.5	1.5
Quality of life				
Baseline	32.2 ± 14.1	30.2 ± 16.2		
Change at 24 months	34.9 ± 22.7	28.4 ± 24.2	7.4	− 1.5
Change at 60 months	40.4 ± 20.3	32.1 ± 27.1	9.1	0.2

Values are presented as mean ± SD or number of patients (% of total)

KOOS Knee injury and osteoarthritis outcome score, *ACI* autologous chondrocyte implantation with spheroids, *CI* confidence interval

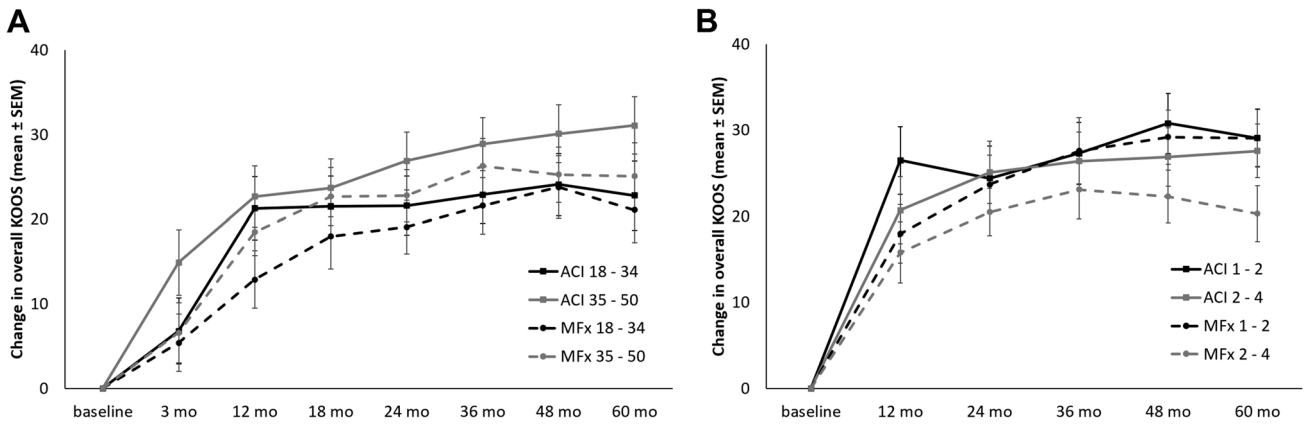

Fig. 3 Improvement in the overall Knee injury and osteoarthritis outcome score (KOOS) compared to baseline for the two treatment groups autologous chondrocyte implantation with spheroids (ACI) and microfracture (MFx) for A) subgroups of 18–34 and 35–50 years of age and B) subgroups of defect sizes 1–2 cm^2 and 2–4 cm^2

mean overall change in KOOS compared to baseline from 12 months onwards (Fig. 3A). For both age groups in the microfracture-treated patients (18 in younger and 31 in older group), most improvement in change in overall KOOS compared to baseline was observed in the first 18 months with clinically relevant improvement compared to baseline from 12 months onwards. After 18 months, the mean change in overall KOOS compared to baseline values remained overall stable (Fig. 3A). Apart from this, no differences could be observed between the two age groups and the two treatment groups, although the improvements in the ACI groups were numerically greater than in the microfracture groups within the respective age groups (Fig. 3A).

Influence of defect size on the overall KOOS

Overall KOOS and KOOS subscores were additionally analyzed for subgroups based on defect size (1–2 cm^2 and > 2 up to 4 cm^2). The subgroup '1–2 cm^2' was represented by 12 patients in the ACI group and 21 patients in the microfracture group, while the subgroup '> 2 up to 4 cm^2' comprised 36 patients treated by ACI, and 27 patients treated by microfracture. The defect size showed no impact on the overall KOOS (Fig. 3B). However, for the larger defects (> 2 up to 4 cm^2) the difference between the change in KOOS between the ACI and microfracture groups became more pronounced from 36 months follow-up onwards.

Clinical outcome: Other

For the IKDC Knee Examination form, an overall improvement for the ACI and microfracture groups was observed (Table 3). At 24 months, 18 ACI patients showed improvement, 27 showed no change (of which 24 already scored A at baseline) and 2 showed worsening. For microfracture, 18 patients showed improvement, 28 showed no change (of which 22 already scored A at baseline) and 3 showed worsening at 24 months. A similar pattern was found at 60 months, except that 3 more ACI patients coming from grade B and C improved to grade A and 3 more microfracture patients coming from grade B and D improved to grade A. For both timepoints, there was no difference between the ACI and microfracture groups.

The IKDC physical component summary showed overall greater improvements in the ACI group compared to the microfracture group (Table 4). No differences were present between the ACI and microfracture groups (Table 4).

The IKDC subjective knee evaluation was increased in both the ACI and microfracture group at 24 and 60 months, but the improvement was higher for the ACI group than the microfracture group at 60 months (Table 4). A similar pattern was observed for the modified Lysholm (24-point scale) (Table 4).

Table 3 Shift table for IKDC grade between baseline and 24- and 60 months follow-up

Grade at baseline	ACI ($n = 48$)				Microfracture ($n = 49$)			
	Grade at 24 months				Grade at 24 months			
	A	B	C	D	A	B	C	D
24 months								
A	24	1	1	0	22	3	0	0
B	9	3	0	0	12	6	0	0
C	8	1	0	0	2	2	0	0
D	0	0	0	0	0	1	1	0
Missing	1	0	0	0	0	0	0	0
Grade at baseline	ACI ($n = 48$)				Microfracture ($n = 49$)			
	Grade at 60 months				Grade at 60 months			
	A	B	C	D	A	B	C	D
60 months								
A	24	1	1	0	22	3	0	0
B	11	1	0	0	13	5	0	0
C	9	0	0	0	2	1	0	1
D	0	0	0	0	2	0	0	0
Missing	1	0	0	0	0	0	0	0

IKDC international knee documentation committee, *ACI* autologous chondrocyte implantation with spheroids

Table 4 Scores for other patient reported outcomes with ACI and microfracture measures

	ACI *N* = *48*	Microfracture *N* = *49*	*P* value ACI vs MFx
IKDC physical component summary			
Baseline	41.6 ± 9.4	38.3 ± 9.0	
24 months	52.8 ± 8.9 ($p < 0.0001$)	47.8 ± 9.6 ($p < 0.0001$)	0.0537
60 months	53.7 ± 7.7 ($p < 0.0001$)	47.3 ± 10.1 ($p < 0.0001$)	**0.0036**
IKDC mental component summary			
Baseline	49.6 ± 9.8	51.0 ± 10.0	
24 months	51.7 ± 11.4 ($p = 0.1198$)	53.6 ± 9.1 ($p = 0.1131$)	0.3746
60 months	53.5 ± 7.9 ($p = 0.0040$)	54.0 ± 8.3 ($p = 0.0352$)	0.6862
IKDC subjective knee evaluation			
Baseline	54.4 ± 15.9	47.8 ± 14.6	
24 months	78.6 ± 16.8 ($p < 0.0001$)	68.3 ± 20.4 ($p < 0.0001$)	0.0788
60 months	84.1 ± 14.3 ($p < 0.0001$)	69.5 ± 19.7 ($p < 0.0001$)	**0.0007**
Modified lysholm*			
Baseline	16.8 ± 4.0	16.0 ± 3.3	
24 months	21.7 ± 2.7 ($p < 0.0001$)	20.4 ± 3.4 ($p < 0.0001$)	0.0811
60 months	22.5 ± 2.4 ($p < 0.0001$)	20.8 ± 3.7 ($p < 0.0001$)	**0.0159**

Bold value indicates $p < 0.05$

*The modified Lysholm has a 24-point scale

ACI autologous chondrocyte implantation with spheroids, *MFx* microfracture, *IKDC* international knee documentation committee

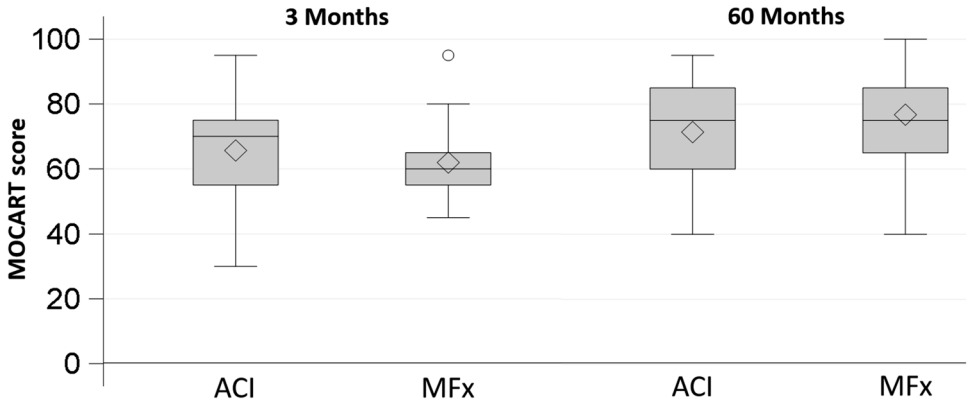

Fig. 4 Magnetic resonance observation of cartilage repair tissue (MOCART) score of patients treated with autologous chondrocyte implantation with spheroids (ACI) and microfracture (MFx) at 3- (first assessment performed) and 60 months follow-up shown in box and whisker plots

Structural repair outcome: MOCART

An improvement was observed for the MOCART score in both the ACI and the microfracture group (Fig. 4). For the ACI group the (mean ± SD) MOCART improved from 65.6 ± 15.5 at 3 months to 71.4 ± 14.8 at 60 months. For the microfracture group it improved from 61.9 ± 10.6 to 76.7 ± 14.9. No difference was found between the ACI and microfracture group. Also for the single items of the MOCART, no differences could be found between the ACI and microfracture group. Even not for the degree of defect fill at the first assessment at 3 months, which scored 14.0 ± 6.1 in the ACI group and 14.2 ± 6.2 in the microfracture group. In addition, no correlation could be observed between the clinical outcome measured by overall KOOS and the MOCART score with Spearman´s coefficients of − 0.006 for the ACI and − 0.078 for the microfracture group at 60 months follow-up.

Safety results

The frequency of adverse events that were classified as probably or possibly related to the study treatment was similar in both treatment groups (Table 5). The most common one found in the ACI group was joint effusion with 24 events in 18 (35%) patients. In the microfracture group, arthralgia was most frequent with 24 events in 18 (36%) patients. In both groups there were 10 patients with joint swelling.

Table 5 Most frequently reported (> 5%*) adverse events classified as probably or possibly related to the study treatment

Treatment group:	ACI $N=52$		Microfracture $N=50$	
	nP (%)	nE	nP (%)	nE
Any	30 (58%)	71	30 (60%)	75
Joint effusion	18 (35%)	24	15 (30%)	18
Arthralgia	10 (19%)	13	18 (36%)	24
Joint swelling	10 (19%)	11	10 (20%)	10
Bone-marrow edema	1 (2%)	1	5 (10%)	5
Contusion	3 (6%)	3	1 (2%)	1

*adverse events that occurred in > 5% of the patients in at least one of the treatment groups

ACI autologous chondrocyte implantation with spheroids, *nP* number of patients, *nE* number of events

A difference was found in the frequency of bone-marrow edema, which occurred five times in five different patients treated with microfracture and only one time in one patient treated with ACI. Three patients treated with ACI had a contusion while this only occurred in one patient treated with microfracture.

In addition, laboratory analyses (hematology and clinical chemistry), vital signs, electrocardiography, body weight and recorded concomitant pain medication taken did not show any sign of safety concern related to either treatment.

Discussion

The most important finding of this study was the maintenance of non-inferiority of ACI with spheroids to microfacture for cartilage defects in the knee at five-year follow-up, while superiority in KOOS subscores "activities of daily living", "quality of life" and "sports and recreation" could be demonstrated at a descriptive level. Results at two and three years of this clinical study have been previously reported, showing relevant clinical improvement, non-inferiority of ACI with spheroids to microfracture and superiority on the descriptive level for a selection of subscores [13, 26].

Here, the final five years results of the prospective clinical study are presented. These results are in agreement with the majority of clinical studies that reported on a comparison of (M)ACI to microfracture. Overall, numerically better clinical improvement or even superiority in one or more clinical outcomes scores in the ACI groups is found at similar follow-up time [1, 17, 29]. Only in comparative studies where first generation ACI is used, no significant advantage of ACI was reported [19]. For a characterized chondrocyte implantation (ChondroCelect (TiGenix)) greater clinical improvement for ACI compared to microfracture was maintained over five years for patients with a symptom duration shorter than three years before treatment [39]. For autologous cultured chondrocytes on porcine collagen membrane (MACI (Sanofi, Genzyme, now Vericel) superiority was shown in the extension study demonstrated at five years follow-up [5]. Moreover, better clinical results were also reported for Hyalograft C over microfracture at five years follow-up [17].

Nowadays, it is generally adopted that ACI is more suitable for the treatment of larger (> 2 cm^2) cartilage defects than microfracture. For this reason, several specialist associations recommend ACI as standard treatment for larger cartilage defects [7, 8, 24]. The findings of the study reported here supports the use of ACI in larger defects. The microfracture treatment of the larger defects in the current study showed a deterioration of the mean change in KOOS compared to baseline after 36 months follow-up. This might also explain why the differences in the change in KOOS between ACI and microfracture in the overall population became more pronounced after 36 months. In addition, based on clinical and safety outcomes, ACI would be suitable for defects that are currently in the indication of microfracture. The improvements in overall KOOS and subscores widely exceed the minimally clinically important difference, showing clear advantages based on one of the most important health outcomes in evaluating the effectiveness of treatments [31, 33]. It would be another discussion whether the improvement would justify the difference in treatment costs between ACI and microfracture.

An important discussion point in using ACI is the age of the patients. It is believed that the regenerative capacities of chondrocytes can be compromised in older patients, due to reduced growth factor signaling abilities. However, it has been previously reported that no difference was found in the treatment of patients younger and older than 40 years of age [25]. Similar findings were found in this study in a subgroup analysis based on age, with an upper age limit of 50 years old. Even more interesting is the finding that the initial improvement is greater in patients between 35 and 50 years of age treated with ACI compared to treatment with microfracture in this age group and patients younger than 35 regardless of the treatment, suggesting that older patients in the currently set ACI age range can benefit at least as much from ACI as younger patients. The general believe that younger patients have a better healing response after microfracture treatment due the increased healing capacities of their bone marrow, could not be confirmed in this analysis where no differences in outcome following microfracture were found.

Structural repair was assessed with MRI, using the MOCART score. For both treatment groups an improvement was found, but no differences between the groups. In addition, no correlation between clinical (KOOS) and radiological (MOCART) outcome was observed. However, this is

consistent with numerous literature reports on ACI and other cartilage repair procedures [10, 42]. One reason for this may be that MRI-based scoring systems aim specifically to score the graft repair rather than the knee in general. A structural repair by ACI or other repair techniques may occur, but general complaints experienced by the patients in the context of the cartilage defect may still exist, even years after treatment, and these may be perceived differently by different patients. This possibility is underlined by the large variation in the KOOS and other patient- reported outcomes at the various times of assessment. However, structural repair is still an important outcome. Although it currently lacks correlation with patient reported outcomes on the short- to midterm follow-up, it might become more important in predicting longer term resilience of repair tissue for certain indications [15] as for instance observed for microfracture [22]. The relatively short follow-up might also be the reason why no differences were found in the MOCART scores between the groups. MRI is still the least invasive procedure to assess structural repair.

The safety findings in this study can confirm the general belief that ACI is a safe procedure that is largely free of complications. Similar to the two years results, the most reported adverse events related to the study treatment were joint effusion, arthralgia and joint swelling. No severe or serious adverse events related to the ACI treatment were reported.

Although the study was designed as a prospective, controlled randomized trial, there are limitations. Firstly, the trial was designed as a non-inferiority study. At the time the study was designed, in 2010, the need for superiority was less clear. In addition, the trial was designed against microfracture. On one hand it is unethical to compare to microfracture while using larger defects as it is known microfracture is not suitable for their treatment [18], on the other hand the sample size would have increased a lot if superiority had to be achieved while using smaller defects. This can also be regarded unethical as at some point an effective treatment needs to be implemented in routine care. However, the fact that superiority at several follow-up times was closely approached and even reached for some KOOS subscores, does emphasize the significance of the results.

Another limitation is that blinding of the subjects was impossible. Also, this would have been unethical and meant that microfracture patients had to undergo two surgeries of which one would have been a sham [4, 41]. Therefore, a risk of bias due to a placebo effect cannot be ruled out. Where possible, analyses were performed by blinded readers such as in the scoring of the MRIs and the biopsies. In addition, LOCF was used to impute data for individual missing scores after at least one post-treatment observation, which may lead to bias of the data. However, especially for the KOOS and MOCART it is known that for ACI most improvement is observed in the first year after treatment, whereafter the values remain stable, even up to 20 years after treatment [6, 29, 43].

Conclusions

Treatment of patients between 18 and 50 years of age with single symptomatic cartilage defects (1–4 cm^2) of the knee with matrix- associated ACI with spheroids provided good improvement in multiple clinical outcome scores up to five years follow-up. Improvement in KOOS and KOOS subscores was non-inferior to treatment with microfracture. Moreover, three important and clinically relevant KOOS subscores showed superiority for treatment with ACI over microfracture. No safety concerns were observed. ACI with spheroids is a safe and effective treatment for single full-thickness cartilage defects of the knee.

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Declarations

Conflict of interest A.H. received royalties for medical consultancy for CO.DON AG. W.Z. has been paid honorary by CO.DON AG for consultant activities. S.F. has received consultant fees from Arthrex and Bauerfeind. P.N. has received grants for educational purposes, including CO.DON AG. Each author has been rewarded with an investigator fee as outlined in the initial clinical trial authorization documents and accepted by the corresponding ethic committees. No further sponsorship was granted.

Ethical approval The trial was approved by the ethics committees responsible for the respective centers and by the local regulatory authorities. The main ethics committee was Mannheim, Germany (2009-070F-MA).

Informed consent Written informed consent was obtained from all participants before the study.

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