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The Effect of Cell Dose on the Early Magnetic Resonance Morphological Outcomes of Autologous Cell Implantation for Articular Cartilage Defects in the Knee

A Randomized Clinical Trial

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Background: Although autologous chondrocyte implantation (ACI) has been established as a standard treatment for large full-thickness cartilage defects, the effect of different doses of autologous chondrocyte products on structural outcomes has never been examined.

Hypothesis: In ACI, the dose level may have an influence on medium-term magnetic resonance morphological findings after treatment.

Study Design: Randomized controlled trial; Level of evidence, 1.

Methods: A total of 75 patients who underwent ACI using a pure, autologous, third-generation matrix-associated ACI product were divided into 3 groups representing different doses: 3 to 7 spheroids/cm^2, 10 to 30 spheroids/cm^2, and 40 to 70 spheroids/cm^2. Magnetic resonance imaging was performed at 1.5, 3, 6, and 12 months after ACI and was evaluated by the magnetic resonance observation of cartilage repair tissue (MOCART) score and the Knee injury and Osteoarthritis Outcome Score (KOOS).

Results: MOCART scores showed improvements after 3 months, with slight dose dependence, and further improvement after 12 months, although without significant dose dependence. The mean MOCART scores after 3 months (0 = worst, 100 = best) were 59.8, 64.5, and 64.7 for the low-, medium-, and high-dose groups, respectively, and 62.9 for all patients; at 12 months, these were 74.1, 74.5, and 68.8 for the respective dose groups and 72.4 for all patients. Several MOCART items (surface of repair tissue, structure of repair tissue, signal intensity of repair tissue, subchondral bone, and synovitis) showed a more rapid response with the medium and high doses than with the low dose, suggesting a potential dose relationship. No significant correlation between the MOCART (overall and subscores) with clinical outcomes as assessed by the overall KOOS was detected at 3- and 12-month assessments.

Conclusion: This study reveals a trend toward earlier recovery after treatment with higher spheroid doses in terms of better defect filling for full-thickness cartilage defects of the knee, while outcomes after 12 months were similar in all dose groups. However, a correlation with clinical outcomes or the failure rate at 1 year after ACI was not found. A longer follow-up will be required for more definite conclusions on the clinical relevance of ACI cell density to be drawn.

Registration: NCT01225575 (ClinicalTrials.gov identifier); 2009-016816-20 (EudraCT number).

Keywords: autologous chondrocyte implantation; cartilage lesion; knee surgery; MOCART; KOOS; randomized clinical trial

Autologous chondrocyte implantation (ACI) was introduced in 1994 by Brittberg et al[5] and has in recent years become established as a standard treatment for large, full-thickness cartilage defects of the knee exceeding 2.5 cm^2 in size.[27] Although several modifications have been introduced, such as the use of various artificial membranes[23] and 2-dimensional pure autologous high-density cell aggregates,[2] all yielding an improvement in clinical outcomes, easier application, and elimination of the need for additional harvesting of the autologous periosteum as initially described by Brittberg et al,[5] the optimum dose in the context of ACI, leading to the best structural and clinical outcomes, has never been investigated thoroughly. Present-day clinical practice, and

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the current recommendation, is to use approximately 1×10^6 to 3×10^6 chondrocytes/cm^2, which is roughly similar to the cell density found in natural adult articular cartilage.[17,41] This is based mainly on expert opinion[40] and clinical experience from sequential case series that led to satisfactory outcomes in this setting.[32-34] In addition, recent prospective randomized trials, conducted to obtain approval of autologous cell products from the European Medicines Agency (EMA), also used cell densities in this range.[37-39] Until now, the cell density to be applied has never been debated thoroughly and is still in line with the cell number recommended by the original investigators.[5] This is also of great importance because it has been described earlier that cell quality affects clinical outcomes.[29,34]

With regard to the preclinical evaluation, some studies have focused on the effect of different cell densities on cartilage repair. Because clinical evidence is lacking, those studies represent the best available evidence concerning the effect of cell density on cartilage regeneration. In addition, studies on the natural development of articular cartilage can serve as examples and models for cartilage regeneration. Those studies have led to the suggestion that an initial cell number that exceeds the cell density of natural adult articular cartilage might be beneficial, mimicking natural chondrogenesis. Some studies on chondrogenesis describe a "condensation" or "aggregation" of migrated chondrogenic precursors as an initial stage of chondrogenesis before matrix synthesis occurs and the cell proliferation rate decreases, leading to a relative decrease in the cell density within the created tissue.[12,13,18,41,42] The hypothesis of a potential benefit of an increased initial cell density is also supported by in vitro tissue-engineering studies in which tissue formation was evaluated following various types of cell implantation on artificial scaffolds, comparable with the clinical setting of ACI. These studies demonstrated (1) a greater amount of cartilage formation with an increased cell number (20,000/mm^2 compared with 5000/mm^2), (2) higher expression levels of cartilage-specific marker genes, (3) the formation of greater amounts of cartilage-associated proteins such as collagen type II or glycosaminoglycans, and (4) an even better morphological structure and biomechanical properties of repair tissue.[7,16,24,35,45] The latter could potentially be related to the observation that a higher density of articular chondrocytes increases the stability of a chondrogenic phenotype and avoids dedifferentiation and may, in this way, contribute to better cartilage formation.[14,30,31] The findings of these studies also explain better tissue formation in later studies in which different cell densities and their influence on tissue formation were compared, with cell densities up to 2.2×10^7/cm^2.[10,17,21] In contrast to this, higher cell densities have also been reported to decrease the production of the extracellular matrix and to down-regulate chondrogenic transcription rates,[1,3,6] while some other studies did not reveal significant differences in tissue formation when different cell densities were compared.[6]

All these observations underline the importance of cell density in cartilage repair and the necessity to conduct clinical studies dealing with its effect on clinical outcomes. Because clinical studies on this are lacking, the present study was initiated to compare the effect of different cell densities and product doses on the quality and structure of repair tissue, as evaluated by sequential morphological magnetic resonance imaging (MRI) investigations up to 1 year after surgery. For this purpose, a prospective, randomized controlled clinical study was designed, comparing 3 different doses using Chondrosphere (co.don AG) in a similar surgical setup. In third-generation matrix-associated chondrocyte products, the cell number is just one of several parameters and does not alone characterize fully the cell product; the matrix, proportion of viable cells, phenotype of the cells, and so on might also contribute significantly to clinical and morphological outcomes. In the present study, the number of spheroids per cm^2 was chosen as the main dose parameter. Doses applied ranged overall from 6 to 84 spheroids/cm^2. The purpose of the study was to assess the short-term efficacy and safety of the different doses in the treatment of large cartilage defects (4-10 cm^2) of knee joints, including specifically the influence of product dose on structural outcomes within the first year after ACI and its correlation with overall clinical outcomes.

METHODS

Study Design and Surgical Treatment

The investigators and sponsor ensured that this study was conducted in full compliance with the protocol, the principles laid down in the Declaration of Helsinki, the Harmonised Tripartite Guideline E6 for Good Clinical Practice of the International Conference on Harmonisation (ICH-GCP), and relevant laws and regulations. The protocol and informed consent form for this study were approved

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in writing by the appropriate ethics committees and the federal authority (Paul-Ehrlich-Institut) in accordance with the laws and regulations of Germany, where the study was conducted, before any participant was included in the study.

This phase 2 study was set up as a single-blinded, prospective, randomized controlled clinical intervention study. The investigators were not blinded. However, the patients were blinded to their spheroid dose level. Blinding of the patients was maintained up to the final assessment and is being maintained during the remaining follow-up period. It was (and remains) crucial that the patients are not informed of the spheroid dose that they have received; any unblinding would lead to withdrawal of the patient from the trial. An independent central radiologist assessed all MRI scans after the study intervention without knowledge of which dose had been applied or the time point (of patient participation in the trial) at which the scan was obtained.

After approval by the local ethics committees and the federal authority and after study registration (ClinicalTrials.gov identifier: NCT01225575; EudraCT No.: 2009-016816-20), patients with symptomatic full-thickness cartilage defects of the knee were included between November 2010 and September 2012 at 10 German orthopaedic centers. In all patients, the indication for study participation was determined during routine arthroscopic surgery of the affected knee joint. Only cartilage defects of International Cartilage Repair Society (ICRS) grade 3 or 4 with a size between 4 and 10 cm^2 were to be included. In all cases, final eligibility was assessed by arthroscopic surgery of the affected knee: only patients with unipolar, focal symptomatic chondral and osteochondral defects with intact adjacent cartilage were included. Inclusion and exclusion criteria are summarized in Table 1. Assignment of the groups is shown in Figure 1.

TABLE 1
Principal Inclusion and Exclusion Criteria[a]

Inclusion Criteria
Age 18-50 years
Isolated, symptomatic full-thickness cartilage defects (ICRS grade 3 or 4)
Osteochondral defects (including osteochondritis dissecans) with bone defects of a maximum 3 mm in depth
Defect location: medial or lateral femoral condyle, trochlea, tibia, and patella; also osteochondritis dissecans (bone grafting up to the level of the original bone lamella was to be performed if bone loss exceeded 3 mm in depth)
Defect size of 4-10 cm^2 after debridement to healthy cartilage
Nearly intact surrounding the chondral structure around the defect as well as the corresponding joint area
Willingness to follow strict rehabilitation protocol and follow-up program
Exclusion Criteria
Defects in both knees at the same time
Radiological signs of osteoarthritis (Kellgren-Lawrence grade 3 or 4)
Ligamentous knee instability
Valgus or varus malalignment (>5° over the mechanical axis)
Clinically relevant second cartilage lesion on the same knee
>50% resection of a meniscus in the affected knee or incomplete meniscal rim
Rheumatoid arthritis, parainfectious or infectious arthritis, or condition after these diseases
Pregnancy and planned pregnancy (no MRI possible)
Obesity (body mass index >30 kg/m^2)
Previous treatment with ACI in the affected knee
Microfracture performed <1 year before screening in the affected knee
Meniscal implant in the affected knee
Meniscal suture (in the affected knee) at 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
Ongoing specific osteoarthritis drugs such as chondroitin sulfate, diacerein, *N*-glucosamine, piascledine, and capsaicin in the 2 weeks before baseline
Corticosteroid treatment by the intra-articular route within 1 month before baseline or systemic (all routes) corticosteroids within 2 weeks before baseline
Chronic use of anticoagulants
Current diagnosis of osteomyelitis, HIV-1 and HIV-2, and/or hepatitis C infection

[a]Complete inclusion and exclusion criteria are provided in the publicly accessible database at https://clinicaltrials.gov/ct2/show/NCT01225575?term=chondrosphere&rank=1. ACI, autologous chondrocyte implantation; ICRS, International Cartilage Repair Society; MRI, magnetic resonance imaging.

Surgical Technique, Assessment, and Rehabilitation

For all patients, cartilage biopsy from the intercondylar notch was performed as described previously.[28] After biopsy and arthroscopic surgery, patients were stratified for randomization into 2 groups according to defect size ($\geq$4 to <7 cm^2 and $\geq$7 to 10 cm^2). After a patient's final inclusion in the study, central randomization via a telephone hotline was performed within each defect-size group: patients were randomly allocated (1:1:1) to treatment groups according to the dose level: low (group L; 3-7 spheroids/cm^2), medium (group M; 10-30 spheroids/cm^2), or high (group H; 40-70 spheroids/cm^2). All patients were blinded to the dose level.

In all groups, during knee arthroscopic surgery, chondrocytes were harvested using a standardized cartilage biopsy tool (Storz) from the intercondylar notch.[28] A total of 3 osteochondral cylinders were harvested in every patient for subsequent cell expansion. Chondrosphere was produced as previously described.[2] The cells were first propagated in a monolayer culture before cultivation as chondrocyte spheroids. Chondrocyte spheroids (Chondrosphere) were generated by seeding 2×10^5 chondrocytes in the 3-dimensional (3D) cell cultivation system, and cultivation was continued. The mean total cultivation duration was 51.4 $\pm$ 7.2 days. The spheroids had a mean diameter of 618 $\pm$ 117 μm at the time of implantation, which was similar for all 3 treatment groups. In group L, the mean spheroid size was 631 $\pm$ 104 μm; in group M, the mean spheroid size was 639 $\pm$ 136 μm and in group H was 583 $\pm$ 105 μm.

After a cell culture period of approximately 8 weeks, in all patients, ACI was performed using a (mini)arthrotomy as the standard approach. Debridement of the cartilage defect into

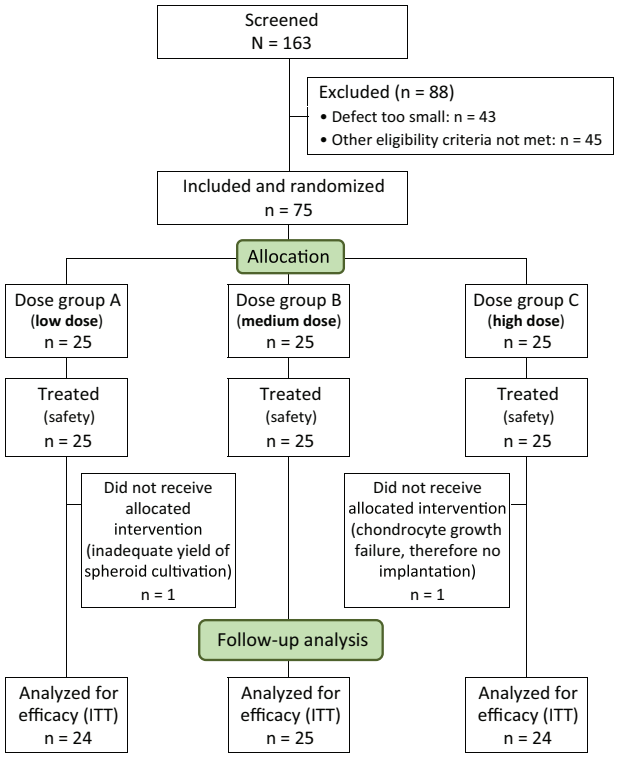

Figure 1. CONSORT flow diagram illustrating patient selection and group assignment. ITT, intention to treat.

the adjacent healthy cartilage was performed, preserving the calcified layer and trying to avoid bleeding from the subchondral bone. After debridement, chondrocyte spheroids were applied at a spheroid dose corresponding to the treatment group assignment. The spheroids were distributed homogeneously within the defect area. After an interval of 20 minutes for adherence of the spheroids, the joint was closed.

After surgery, a standardized rehabilitation protocol was applied, which included continuous passive motion from the day after surgery for 6 weeks (up to 4 h/d). Limitations on weightbearing for 6 weeks were recommended. It was increased to full weightbearing between 6 and 8 weeks after surgery. Individual limits of flexion were also given, depending on the exact defect location, to avoid early exposure of the regenerative cartilage to axial compression and shear forces.

The MRI-based magnetic resonance observation of cartilage repair tissue (MOCART) scoring system allows the evaluation, analysis, and description of 3 clinical variables: (1) the radiological outcome of matrix-associated chondrocyte transplantation several months or even years after surgery, (2) the statistical description of the interobserver variability, and (3) the statistical correlation of the subjective clinical outcome with the radiological variables of the MRI scoring system.[22,46] The MOCART scoring system ranges from 0 (worst possible case) to 100 (normal), where "worst possible case" means exposed subchondral bone, including incomplete integration to the border zone, damaged surface of the repair tissue, inhomogeneous structure of the repair tissue or cleft formation detected, no intact subchondral lamina, adhesions, synovitis, and subchondral bone with edema, granulation tissue, cysts, or sclerosis. MRI scans were assessed centrally by a blinded reader, and assessments were performed centrally by a blinded radiologist. Because of the patients' clinical condition, MOCART scores could not be measured at baseline, but for the same reason (cartilage defect with ICRS grade 3 or 4), a value close to 0 could reasonably be imputed. A representative MRI follow-up is shown in Figure 2.

Safety was analyzed by the tabulation of adverse events (numbers of reports and numbers/percentages of patients affected) and by descriptive statistics for vital signs, body weight and body mass index, and standard laboratory variables. All documentation of the study including the definition of adverse events was performed in accordance with good clinical practice guidelines (ICH-GCP). Clinical investigations and evaluation of the outcome scores were performed by the principal investigator of the individual center. Patients were blinded to group assignment during the entire follow-up period.

Statistical Analysis

The primary analysis was performed according to a prospectively defined hierarchical scheme: First, the primary efficacy variable at the final assessment was compared with its baseline value (ie, before arthroscopic surgery) for group H; the same comparison was next made for group M and then for group L. Finally, an exploratory between-group comparison was performed. The primary analysis set comprised all the patients treated (intention to treat); a supporting per-protocol analysis did not yield notably different results and is not described here.

For correlation with clinical outcomes, results on the overall Knee injury and Osteoarthritis Outcome Score (KOOS) were evaluated. Analysis of variance was used to detect significant differences between different time points and for between-group testing. Analogous secondary analyses were performed, at a descriptive level, where the structure of the variable allowed this; in other cases, appropriate descriptive statistics were provided. The potential correlation between KOOS and MOCART results at different time points was investigated by calculating the Spearman correlation coefficient for all patients for whom both KOOS and MOCART results were available.

Statistical analyses were performed by StatConsult GmbH. Before enrollment, a sample size calculation was performed based on the following assumptions: test for superiority at follow-up to baseline (day 0) with respect to the KOOS, $\alpha = .05$, 2-sided testing, minimal relevant difference of 12.5%, and power of 0.90. Following these assumptions, a minimum of 18 patients per group was needed (without dropouts).

RESULTS

Patient Population

The dose groups were well balanced with respect to size, demographics, and disease background; minor imbalances

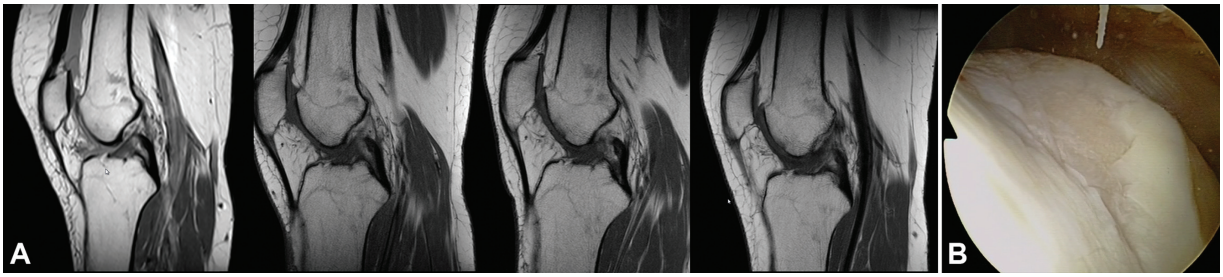

Figure 2. (A) Typical magnetic resonance imaging follow-up of a patient treated for a cartilage defect of the medial aspects of the trochlea (from left to right: preintervention, and 3-month, 12-month, and 24-month follow-ups). (B) Arthroscopic image.

TABLE 2
Patient Demographic and Baseline Data[a]

	Dose Group			
	L (3-7 spheroids/ cm^2; n = 25)	M (10-30 spheroids/ cm^2; n = 25)	H (40-70 spheroids/ cm^2; n = 25)	All Patients (N = 75)
Sex, n				
Female	8	4	10	22
Male	17	21	15	53
Age, mean ± SD, y	33 ± 10	34 ± 9	34 ± 9	34 ± 9
BMI,* mean ± SD (range), kg/m^2	24.9 ± 2.5 (21.3-29.8)	25.6 ± 3.2 (19.4-33.2)	25.1 ± 3.6 (19.0-32.3)	25.2 ± 3.1 (19.0-33.2)
Smoking, n				
Yes	8	4	6	18
No	7	21	19	47
Diagnosis, n				
Trauma	14	14	9	37
Other	8	6	10	24
OA	2	2	3	7
OD	1	3	3	7
Defect size, mean ± SD (range), cm^2	4.8 ± 1.5 (0.5†-7.5)	4.9 ± 1.3 (1.3†-7.5)	5.2 ± 1.3 (3.0†-8.0)	5.0 ± 1.3 (0.5†-8.0)
Primary defect location, n				
Femur	9	10	9	28
Tibia	—	—	—	—
Patella	16	15	16	47
Presence of further defects (ICRS grade >3), n				
Femur	2	1	—	3
Tibia	1	1	1	3
Patella	2	1	1	4

[a]Minor imbalances observed for sex and smoking were not statistically different as determined by the Fisher exact test. BMI, body mass index; H, high dose; ICRS, International Cartilage Repair Society; L, low dose; M, medium dose; OA, osteoarthritis; OD, osteochondritis dissecans.

(patient sex, smoking habit) were not considered relevant as these imbalances were not statistically different in the 3 treatment groups. The analysis population comprised 75 patients (22 women, 53 men) aged 34 ± 9 years. Twenty-five patients were assigned to each treatment group. The follow-up rate was 96% (n = 24) in groups L and H and 100% in group M. The medical history at baseline was unremarkable except for a greater number of patients with a history or presence of "infections and infestations" in group M. Primary defect locations were mostly the patella (47/75) or the femur (28/75); the tibia was not represented. ICRS grades were mostly 3C or 4A and were fairly evenly distributed between the treatment groups.

Defect sizes ranged from 0.5 to 8.0 cm^2 after intraoperative debridement (2 patients were included with a defect that turned out to be <4 cm^2 in size; these were nonetheless treated and were therefore retained for the intention-to-treat analysis). Apart from this, and from the administration of lower doses than envisaged to 10 patients in group H because of inadequate cell proliferation, compliance with the study treatment and with the subsequent rehabilitation measures (which were completed by 72/75 study patients) was good. Demographic and baseline data are summarized in Table 2.

Efficacy Results: Clinical

For the evaluation of clinical outcomes, KOOS values were assessed. The overall KOOS value is measured on a scale

TABLE 3
KOOS Values at 12 Months After Implantation[a]

	Dose Group			
	L (n = 24)	M (n = 25)	H (n = 24)	All (N = 73)
Baseline	60.4 ± 13.6	59.6 ± 15.4	51.1 ± 15.4	57.0 ± 15.2
12 months	77.8 ± 14.1	76.3 ± 11.5	65.3 ± 23.2	73.2 ± 17.6
Change	17.4 ± 18.5	16.7 ± 17.9	14.2 ± 18.5	16.2 ± 18.1
P value (*t* test)	.0002	.0001	.0010	—

[a]Data are reported as mean ± SD. Differences from total population sizes are caused by missing results. H, high dose; KOOS, Knee injury and Osteoarthritis Outcome Score; L, low dose; M, medium dose.

TABLE 4
MOCART Scores After Implantation[a]

	Dose Group							
	3 Months After Implantation				12 Months After Implantation			
	L (n = 20)	M (n = 19)	H (n = 19)	All (N = 58)	L (n = 22)	M (n = 19)	H (n = 21)	All (N = 62)
Mean ± SD	59.8 ± 10.9	64.5 ± 10.3	64.7 ± 9.4	62.9 ± 10.3	74.1 ± 13.0	74.5 ± 14.5	68.8 ± 11.4	72.4 ± 13.0
Minimum	45	50	45	45	55	50	45	45
Lower quartile	53	60	60	60	60	60	65	60
Median	60	60	65	60	75	75	70	70
Upper quartile	68	75	70	70	85	90	75	80
Maximum	85	85	90	90	95	100	100	100

[a]Differences from total population sizes are caused by missing results. H, high dose; L, low dose; M, medium dose; MOCART, magnetic resonance observation of cartilage repair tissue.

from 0 (worst) to 100 (best). Results after 12 months compared with baseline are shown in Table 3. A general improvement was clear, and the similarity in responses between the 3 dose groups was manifest in the similar differences between the baseline and 12-month assessments. There was no clear relationship between dose and effect: the comparison between the 12-month and baseline assessments gave *P* values well below the conventional significance threshold of .05 in all 3 dose groups, while none of the between-group comparisons gave significant values ($P > .6$ throughout). A complete analysis of the KOOS results will be published in a subsequent longer term study.

Efficacy Results: MOCART

MOCART scores were acquired at study visits 3 and 12 months after implantation. Baseline MOCART scores were taken to be close to zero. Results are presented in Table 4. Results in all treatment groups showed a similar trend: an increase from the putative low baseline value to the 3-month visit, and a further increase to the 12-month visit, in the sense that the effect of the smallest doses of chondrocyte spheroids took effect the most slowly and the highest doses the most quickly. The difference between the values at 3 and 12 months was highest for group L, suggesting that the low-dose treatment may take effect more slowly and thus indicate the possible existence of a dose relationship (Figure 3). For both the femur and patella, overall MOCART results were consistently high at visits 3 and 12 months after treatment (femur, 75.0 and 72.8, respectively; patella, 71.0 and 70.2, respectively). The results for femoral and retropatellar defects were comparable.

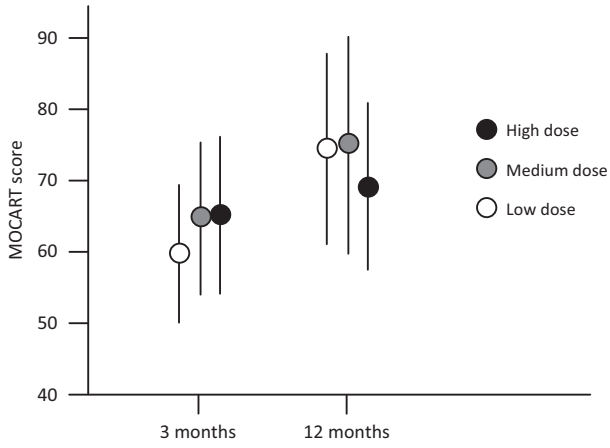

Figure 3. Magnetic resonance observation of cartilage repair tissue (MOCART) score after 3 and 12 months. The circles indicate means, and the bars indicate SDs.

Corresponding results are shown for the separate MOCART items in Table 5. As was found for the total

TABLE 5
MOCART Items After Implantation[a]

Item	Maximum Score for Item	MOCART Score by Dose Group							
		3 Months After Implantation				12 Months After Implantation			
		L (n = 20)	M (n = 24)	H (n = 21)	All (N = 65)	L (n = 22)	M (n = 24)	H (n = 21)	All (N = 67)
1. Degree of defect repair and filling	20	14.8 ± 5.5	13.8 ± 5.4	14.3 ± 5.8	14.2 ± 5.5	15.7 ± 4.4	17.5 ± 4.4	15.5 ± 4.7	16.3 ± 4.6
2. Integration to border zone	15	14.0 ± 2.6	14.8 ± 1.0	14.8 ± 1.1	14.5 ± 1.7	14.1 ± 2.0	14.8 ± 1.0	14.0 ± 2.6	14.3 ± 1.9
3. Surface of repair tissue	10	8.0 ± 2.5	9.6 ± 1.4	8.3 ± 2.9	8.7 ± 2.4	9.3 ± 1.8	9.2 ± 1.9	9.3 ± 1.8	9.3 ± 1.8
4. Structure of repair tissue	5	2.8 ± 2.6	4.0 ± 2.1	4.0 ± 2.0	3.6 ± 2.3	3.6 ± 2.3	4.2 ± 1.9	2.9 ± 2.5	3.6 ± 2.3
5. Signal intensity of repair tissue: dual T2-FSE	15	3.8 ± 2.2	5.6 ± 4.7	4.0 ± 3.4	4.5 ± 3.7	10.5 ± 5.1	9.2 ± 5.6	7.9 ± 4.6	9.2 ± 5.2
6. Signal intensity of repair tissue: 3D-GE-FS	15	4.3 ± 4.4	4.4 ± 1.7	5.2 ± 3.7	4.6 ± 3.3	6.6 ± 5.0	6.0 ± 4.4	5.7 ± 3.3	6.1 ± 4.2
7. Subchondral lamina	5	4.8 ± 1.1	4.4 ± 1.7	4.3 ± 1.8	4.5 ± 1.6	4.8 ± 1.1	4.6 ± 1.4	4.3 ± 1.8	4.6 ± 1.4
8. Subchondral bone	5	1.0 ± 2.1	1.3 ± 2.3	2.3 ± 2.6	1.5 ± 2.3	1.6 ± 2.4	2.6 ± 2.6	1.9 ± 2.5	2.0 ± 2.5
9. Adhesions	5	5.0 ± 0.0	4.8 ± 1.0	4.5 ± 1.5	4.8 ± 1.1	4.8 ± 1.1	4.6 ± 1.4	4.5 ± 1.5	4.6 ± 1.3
10. Synovitis	5	1.5 ± 2.4	2.5 ± 2.6	1.7 ± 2.4	1.9 ± 2.5	3.2 ± 2.5	2.9 ± 2.5	2.9 ± 2.5	3.0 ± 2.5

[a]Data are reported as mean ± SD. Differences from total population sizes are caused by missing results; thus, the sample sizes were the same as, or in some cases greater than, those in Table 4. 3D-GE-FS, 3-dimensional gadolinium-enhanced and fat saturation; H, high dose; L, low dose; M, medium dose; MOCART, magnetic resonance observation of cartilage repair tissue; T2 FSE, T2-weighted fast spin echo.

MOCART score, all items showed an increase compared with the putative low value at baseline in all treatment groups. In some cases (items 2, 3, 7, and 9), they approached the respective best value possible and thus provided prima facie support for the efficacy of the study treatment. For items 2, 7, and 9, no difference between the 3 treatment groups was seen, with all 3 groups showing approximately comparable results at both visits (3 and 12 months after implantation). For items 2, 3, 4, 5, 6, 8, and 10, the 3-month improvement in group L was smaller than that in groups M and H. This difference was no longer clear after 12 months. Thus, several MOCART items (surface of repair tissue, structure of repair tissue, signal intensity of repair tissue, subchondral bone, and synovitis) showed a more rapid response with the medium and high doses of chondrocyte spheroids than with the low dose, implying a possible treatment difference (potential dose relationship) for these items only.

In summary, the overall MOCART assessment revealed improvements after 3 months, with slight dose dependence, and a further improvement after 12 months, although without dose dependence. Several MOCART items, as listed above, showed a more rapid response with the medium and high doses of chondrocyte spheroids than with the low dose, implying a potential dose relationship.

Interestingly, no statistical correlation in any dose groups was found between MRI outcomes as assessed by the MOCART score and overall KOOS value or any KOOS subscores at 12 months after treatment, except for a slight negative correlation of the overall KOOS, KOOS-symptoms, and KOOS-pain with the MOCART at 3 months in group H (details including significances are provided in Table 6). Values of the Spearman coefficient (ρ) were found to lie between -0.534 and 0.418.

Safety Results

All patients received a sufficient dose of chondrocyte spheroids to allow a meaningful assessment of the product's safety. The absolute dose range was 28 to 290 spheroids. The overall incidence of adverse events, of patients with any adverse events, and of patients with treatment-related adverse events did not differ substantially between the treatment groups; no dose relationship was detected. There were 2 severe adverse events, both joint effusion: one in group M, considered probably related to the study treatment, and one in group H, possibly treatment related. There were also 2 serious adverse events, convulsion and arthralgia, both in group L and both considered unrelated to the study treatment.

DISCUSSION

The purpose of the present study was to evaluate the effect of different doses of the third-generation matrix-associated autologous chondrocyte cell product (Chondrosphere) on the structure, amount, and quality of repair tissue within the first year after ACI. The assessment included a serial MRI investigation. As experience with the dose range of 10 to 70 spheroids/cm^2 had been available since 2004, the present study was also intended to confirm that clinical efficacy (measured by the KOOS and other scores) and structural filling (measured by MRI and structural scores) do not differ within this dose range or in time. Therefore, one group received 10 to 30 spheroids/cm^2, and the second group received 40 to 70 spheroids/cm^2. In addition, a third dose group (3-7 spheroids/cm^2) was examined to establish a minimum effective dose. The most important finding of

TABLE 6
Correlation of MOCART and KOOS Outcomes at 3 and 12 Months After Implantation[a]

	n	Spearman ρ / *P* Value > |*r*|	MOCART	KOOS Overall	KOOS Subscale				
					Pain	Symptoms	ADL	Sports/Rec	QoL
MOCART: L group									
3 months	20	ρ	1.0000	–0.0738	–0.0964	0.0127	–0.0668	–0.0341	0.0589
		P value	—	.7641	.6861	.9577	.7860	.8898	.8050
12 months	22	ρ	1.0000	0.2669	0.0096	0.3865	0.1615	0.2239	0.1480
		P value	—	.2554	.9672	.0835	.4963	.3291	.5221
MOCART: M group									
3 months	19	ρ	1.0000	0.4177	0.1910	0.1866	0.1101	0.3311	0.2378
		P value	—	.1074	.4477	.4443	.6741	.1661	.3270
12 months	17	ρ	1.0000	0.2315	0.1273	–0.2416	–0.0587	0.2998	0.2916
		P value	—	.3712	.6263	.3502	.8230	.2423	.2560
MOCART: H group									
3 months	19	ρ	1.0000	–0.4562	–0.5337	–0.4578	–0.4271	–0.0843	–0.3610
		P value	—	.0496	.0186	.0487	.0682	.7315	.1289
12 months	20	ρ	1.0000	–0.1850	–0.2573	–0.0126	–0.3041	–0.1170	–0.2444
		P value	—	.4483	.2877	.9593	.2056	.6334	.3133

[a]ADL, activities of daily living; H, high dose; KOOS, Knee injury and Osteoarthritis Outcome Score; L, low dose; M, medium dose; MOCART, magnetic resonance observation of cartilage repair tissue; QoL, quality of life; Sports/Rec, sports and recreation.

the present study was that, in spite of a more than 7-fold difference in the chondrocyte dose per spheroid, no clear advantage in favor of higher doses with regard to early magnetic resonance tomography outcomes was found.

Hypothetically, lower doses may reach suboptimal therapeutic levels, leading to incomplete defect filling and progression to osteoarthritis or even the need for joint replacement. Thus, because of medical and ethical considerations, the dose range of 3 to 7 spheroids/cm^2 was chosen as the lowest dose range for this dose-response study.

For ACI suspension products, approximately 1×10^6 chondrocytes/cm^2 is currently considered to make up the lowest effective dose, as recommended by the Joint Advisory Board of the German Society for Traumatology (DGU) and the German Society for Orthopaedics and Orthopaedic Surgery (DGOOC)[27] and diverse experts[40] and as currently used for various autologous chondrocyte products including ChondroCelect (Tigenix). ChondroCelect was the first autologous chondrocyte product (a cell-suspension product) in Europe, obtaining marketing authorization from the EMA under the new legislation.[38,39,43,44] In the meantime, a second product of this kind, MACT (MACI by Genzyme), has been authorized by the EMA.[37] Nevertheless, for matrix-associated ACI products, experience with cell-suspension products indicates that the effectiveness of the product is not determined by the cell number alone. Although the manner in which the matrix and cells interact has not yet been systematically elucidated, and it is not fully known what factors are ultimately responsible for the regeneration of cartilage tissue, there is nonetheless substantial clinical evidence[11,15,25,47] that the 3D arrangement—on biomaterials and in autologous pellet cultures—stabilizes the chondrogenic phenotype and may thus also contribute to the regenerative capacity of the implanted cells. Against this background, it appeared reasonable to define the dose level not in terms of the number of cells transplanted from the culture but rather in terms of the quantity of the implanted cell/matrix material. This consideration was reinforced by the fact that the positive influence of the regenerative capacity of cartilage cells and their vitality and phenotype on clinical results has already been demonstrated.[29,34] In chondrocyte spheroids, the chondrocytes are embedded in a 3D scaffold, resulting in an equal distribution of the cells and thus in improved proliferation, differentiation, migration, and attachment of the cells. The 3D structure of chondrocyte spheroids results in an enhanced differentiation of the cells, which start to produce an endogenous extracellular matrix. This endogenous extracellular matrix contains extracellular matrix proteins specific for hyaline cartilage such as proteoglycans and has therefore a hyaline-like character.[2] No xenogenous scaffold is present in chondrocyte spheroids. The cells present in chondrocyte spheroids are already in a chondrogenic state and start to produce the extracellular matrix already before implantation,[2] which they continue to produce on implantation into the cartilage defect.

Therefore, the present prospective, randomized controlled clinical trial was initiated to compare 3 different levels of a spheroid dose in patients with isolated, full-thickness cartilage defects of the knee ranging in size from 4 to 10 cm^2. Except for the doses administered, the treatments were identical, allowing the effect of the cell number on outcome parameters to be studied in isolation. The patients were blinded to the cell density that they received; however, because surgeons obviously need to ensure that the number of spheroids administered corresponded to the patient's group assignment, a double-blind study design was impossible. Nevertheless, the MRI evaluation was performed by independent investigators blinded to the group assignment, allowing an unbiased assessment of structural repair.

Even though there is currently controversy over the relation between MRI findings and clinical outcomes,[19,20] MRI still represents the gold standard for noninvasive monitoring of repair tissue after cartilage repair. In the present study, the MOCART score was used for qualitative assessment of repair tissue by MRI, as introduced for the specific purpose of examining cartilage repair tissue and as recommended and used by various authors.

Interestingly, even though the MOCART score represents the best standardized score for the postsurgical evaluation of repair tissue after cartilage repair interventions, only a limited correlation between the MOCART score and clinical outcomes was found in various studies and independent meta-analyses in recent years.[4,8,9,36] This observation corresponds to that made in further high-quality studies that also failed to find a correlation between standardized outcome measurements, such as the KOOS, and the overall MOCART score,[9] and it also corresponds to the results of the present study.

Nevertheless, independently of the overall MOCART score, there are some significant results in the literature that indeed demonstrate a better correlation between the items of the MOCART score and clinical outcomes, suggesting their possible value as prognostic factors. While Ebert et al[9] found an association between the parameters of effusion and subchondral lamina and KOOS subscores years after matrix-induced ACI, Mithoefer et al[26] found a correlation between the MRI parameter of defect filling and clinical outcomes in a prospective cohort study of a group of patients who underwent arthroscopic microfracturing for symptomatic cartilage defects of the knee. Furthermore, incomplete filling of the defect was also found by Knutsen and coworkers[19,20] to be associated with inferior clinical outcomes in the first prospective, randomized controlled clinical trial comparing the outcomes of arthroscopic microfracturing and first-generation ACI. Because the principle of microfracturing and first-generation ACI is similar to that of the technique used in the present study (all representing in situ regeneration techniques in which repair tissue is supposed to grow after initial cell attachment to the subchondral bone plate), it seems likely that this effect will also be of clinical relevance in third-generation and other modern ACI systems. In addition, those studies underline the usefulness of individual items of the MOCART score for the evaluation of repair tissue after ACI. In the present study, as a major result concerning the morphological evaluation of repair tissue by MRI, a small trend toward improved repair tissue with a higher cell density was found at 3 months after ACI, which disappeared over time until no differences could be observed in the MRI evaluation at 12 months after ACI. Nevertheless, even this effect lacked statistical significance.

Although the present study presents new data on potential dose effects in ACI, there are certainly some limitations of the present study. First is the limited follow-up period of only 12 months. It certainly seems appropriate to study first morphological effects based on an MRI analysis in this short period of time; nevertheless, a longer MRI and clinical follow-up might reveal further differences and insight information. As in every clinical trial, the second issue is potential bias. In the present study, the treating orthopaedic surgeons were not blinded, so a bias might occur. In contrast, the radiologist who performed the MRI analysis by means of the MOCART was blinded, so this error seems limited. A third limitation might be patient selection because of the strict inclusion and exclusion criteria. This is a potential limitation for the vast majority of clinical trials, and all conclusions of the present study are limited to this highly selected subgroup of patients undergoing cartilage repair.

Independent of these limitations, in summary, the data presented here suggest that as regards clinical outcomes, with a focus on the postsurgical objective evaluation of repair tissue by MRI, all cell doses used in the present study led to satisfactory and reliable outcomes, demonstrating the overall efficacy of all dose levels used, even in the low-dose group. Furthermore, no dose-dependent side effects were observed. A detailed examination of the data, including an analysis of individual MRI items, suggests a potential advantage of higher cell doses in terms of faster recovery without being able to demonstrate statistical significance. A clinical correlation for this interesting observation is still lacking, and further evidence with a longer term follow-up is needed. Furthermore, a more detailed characterization of variation among implanted spheroids and their potential influence on clinical outcomes will be of great importance.

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