Autologous Chondrocyte Implantation Using the Original Periosteum-Cover Technique Versus Matrix-Associated Autologous Chondrocyte Implantation: A Randomized Clinical Trial

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Autologous Chondrocyte Implantation Using the Original Periosteum-Cover Technique Versus Matrix-Associated Autologous Chondrocyte Implantation

A Randomized Clinical Trial

Felix Zeifang,* MD, PhD, Doris Oberle, PhD, Corinna Nierhoff, MD, Wiltrud Richter, PhD, Babak Moradi, MD, and Holger Schmitt, MD, PhD
From the Orthopädische Universitätsklinik Heidelberg, Heidelberg, Germany

Background: Autologous chondrocyte implantation (ACI) is frequently used to treat symptomatic defects of the articular cartilage.

Purpose: To test whether matrix-associated autologous chondrocyte implantation or the original periosteal flap technique provides superior outcomes in terms of clinical efficacy and safety.

Study Design: Randomized controlled trial; Level of evidence, 2.

Methods: Twenty-one patients (mean age, 29.3 ± 9.1 years) with symptomatic isolated full-thickness cartilage defects (mean 4.1 ± 09 cm^2) at the femoral condyle were randomized to matrix-associated autologous chondrocyte implantation or the original periosteal flap technique. The primary outcome parameter was the postoperative change in knee function as assessed by the International Knee Documentation Committee (IKDC) score at 12 months after ACI. In addition, the IKDC score was assessed at 3, 6, 12, and 24 months after surgery. Secondary outcome parameters were postoperative changes in health related quality of life (Short Form-36 Health Survey), knee functionality (Lysholm and Gillquist score), and physical activity (Tegner Activity Score) at 3, 6, 12, and 24 months after ACI. Magnetic resonance imaging was performed to evaluate the cartilage 6, 12, and 24 months after ACI and rated using the Magnetic Resonance Observation of Cartilage Repair Tissue score. Adverse events were recorded to assess safety.

Results: The primary outcome parameter showed improvement of patients 1 year after autologous chondrocyte implantation, but there was no difference between the periosteal flap technique and matrix-associated ACI ($P = .5573$); 2 years after ACI, a similar result was found ($P = .4994$). The study groups did not show differences in the Short Form-36 categories and in knee functionality as assessed by Tegner Activity Score 12 months ($P = .4063$) and 24 months ($P = .1043$) after ACI. There was a significant difference in the Lysholm and Gillquist score at 12 months ($P = .0449$) and 24 months ($P = .0487$) favoring the periosteal flap technique group. At 6 months after surgery, a significantly lower Magnetic Resonance Observation of Cartilage Repair score was obtained in the matrix-associated ACI group ($P = .0123$), corresponding to more normal magnetic resonance imaging diagnostic findings. Twelve and 24 months after ACI, the differences between the 2 groups were not significant (12 months, $P = .2065$; 24 months, $P = .6926$). Adverse events were related to knee problems such as transplant delamination, development of an osseous spur, osteochondral dissection, and transplant hypertrophy. Systemic (allergic, toxic, or autoimmune) reactions did not occur.

Conclusion: There was no difference in the efficacy between the original and the advanced ACI technique 12 and 24 months after surgery regarding International Knee Documentation Committee, Tegner Activity Score, and Short Form-36; however, with respect to the Lysholm and Gillquist score, better efficacy was observed in the periosteal flap technique group.

Keywords: autologous chondrocyte implantation; cartilage defect; femoral condyle; periosteal flap; matrix-associated

*Address correspondence to Felix Zeifang, MD, PhD, Orthopädische Universitätsklinik Heidelberg, Schlierbacher Landstrasse 200 A, 69118 Heidelberg, Deutschland (e-mail: felix.zeifang@ok.uni-heidelberg.de).

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Hyaline cartilage has only a very restricted capability of regeneration in the adult.[7] Among other orthopaedic techniques such as bone-marrow stimulation or osteochondral grafting, autologous chondrocyte implantation (ACI) is a clinically well-established treatment option for articular cartilage repair. Autologous chondrocyte implantation was developed and described by Brittberg et al[5] in 1994. Subsequently, randomized clinical trials (RCTs) have

shown the clinical efficacy of ACI for the treatment of cartilage defects.[4,23] Autologous chondrocyte implantation is indicated for extended cartilage defects of Outerbridge grades[37] III and IV, sized 3 to 10 cm^2.[43]

Autologous Chondrocyte Implantation With Periosteal Flap (ACI-P): ACI of the First Generation

According to the original ACI technique, the cartilage defect is covered by a periosteal flap removed from the tibia (ACI-P). After this bioactive chamber is filled it is sealed by fibrin glue. Despite good clinical results, ACI-P has some disadvantages. To obtain the periosteal flap for covering the defect, a second surgical procedure at the tibia is required, causing additional pain and risks for the patient. In addition, the periosteal flap has to be fixed by suture, thus damaging healthy cartilage. Further disadvantages are low mechanical stability as well as the fact that chondrocytes may be expressed from the defect cavity beneath the periosteal flap with minimal physical stress.[39] The chondrocytes disperse unequally in the lesion zone because of gravity.[39] Furthermore, hypertrophy, delamination, or even transplant failure may occur.[44]

Autologous Chondrocyte Implantation Using a Flap Made of Collagen (ACI-C): ACI of the Second Generation

To avoid removal of periosteum from the tibia, a more advanced ACI technique was developed. Instead of a periosteal flap, a membrane of porcine type I/III collagen was used (ACI-C) to cover the lesion filled with cultured chondrocytes.[1,2,4,12] A major advantage of the ACI-C method compared with first-generation ACI was a lower incidence of graft hypertrophy while obtaining similar clinical results.[4,12] A prospective study with a follow-up of a relatively large study population (n = 63) for over 3 years after ACI-C showed that hypertrophy can be avoided by using a membrane made of type I/III porcine collagen.[40]

Matrix-Associated ACI (m-ACI): ACI of the Third Generation

Despite the short- and intermediate-term results of first- and second-generation ACI being favorable, resources are being directed toward research to improve the technology. Problems in the cultivation of chondrocytes are the slow growth and the dedifferentiation of cells, including a switch of collagen synthesis from type II to type I. By adding specific growth factors like transforming growth factor–β to the culture medium monolayer, cultivated cells could be stimulated to reexpress a differentiated phenotype and to produce extracellular matrix.[11] The authors hypothesized that transforming growth factor–β may play a role in the development of cellular aggregations, the initiation of cell proliferation, and the development of hyaline cartilage.

Analysis of gene expression[20] showed that during the cultivation in monolayer, chondrocytes lose their typical characteristics and dedifferentiate. On a molecular level, losing the chondrocyte-specific phenotype is characterized by repression of the cartilage-specific type II collagen and the induction of a fibroblast-specific type I collagen. Besides type II collagen, also the cartilage-specific genes for cartilage oligomeric matrix protein, cartilage link protein, and aggrecan are repressed. The reinduction of a chondrocyte-specific phenotype is obtained by 3-dimensionally arranging the dedifferentiated chondrocytes.

Gigante et al[10] studied the distribution, vitality, and phenotype of chondrocytes cultivated on a 3-dimensional (3D) matrix at the time of implantation using remains of the implanted bioscaffold. In all samples, they found a large number of vital, relatively homogeneously distributed cells that seemed to be well differentiated. Under the scanning electron microscope, these chondrocytes were tightly attached to the matrix and had a spherical shape as well as a rough surface. They reacted with anti–S-100 protein as well as with antibodies against collagen type II and chondroitin-S, indicating that the cells had the characteristics of differentiated cells at the time of implantation.

To verify cartilage ripening of 3D-packed chondrocytes in vivo, transplants were implanted subcutaneously in homozygous, naked mice with aplasia of the thymus.[20] The explanted cartilage tissue of the killed mice subsequently underwent histologic and immunohistochemical analyses. Histologic analysis revealed a uniform distribution of transplanted chondrocytes. Staining with Alcian blue indicated the synthesis and homogeneous accumulation of intercellular proteoglycans belonging to an extracellular matrix. Collagenous parts of the matrix were detected by staining according to Masson-Goldner (pericellular) staining. Within the extracellular matrix, type II collagen was homogeneously distributed, enclosing the chondrocytes and thus confirming the development of hyaline-like cartilage in the naked mouse model.

Considering the obvious advantages of the 3D cultivation, there is a trend toward cultivating chondrocytes on a 3D bioscaffold.

Matrix-associated ACI, a refinement of ACI,[3] has been on the market only for a short time in Europe. Instead of a chondrocyte-containing fluid, a bioscaffold "matrix" is embedded with autologous chondrocytes and implanted with fibrin glue. The following scaffolds were tested in animal experiments: demineralized bone, bioactive glass, fibrin, carbon, and nets of hyaluronan. In clinical use are polymers (eg, BioSeed, BioTissue Technologies, Freiburg im Breisgau, Germany), hyaluronan (eg, Hyalograft C, Fidia, Advanced Polymers, Abano Terme, Italy), collagen sponges (MACI, Verigen, Leverkusen, Germany; Chondro-Guide, Geistlich Pharma, Wolhusen, Switzerland; Novocart, Tetec, Reutlingen, Germany; and Arthromatrix, Orthogen, Düsseldorf, Germany) and collagen gels (eg, CaReS, Ars Arthro, Esslingen, Germany; and Atellocollagen, Koken, Tokyo Japan).

A clinical superiority of any m-ACI product in comparison with first- or second-generation ACI has not been demonstrated. This prospective RCT was initiated to compare

m-ACI and ACI-P in terms of clinical efficacy and safety in the treatment of traumatic and traumatic-degenerative articular cartilage defects of the knee.

MATERIALS AND METHODS

Inclusion criteria were patient age between 16 and 50 years, with isolated cartilage defects between 2.5 and 6 cm^2 detected by MRI and verified with arthroscopy and localized at the medial or lateral femoral condyle. Exclusion criteria were extended cartilage erosion, restricted mobility, corresponding cartilage defects >grade II according to Outerbridge[37] (ie, "kissing" defects or defects on the opposing surface), extended meniscal defect (meniscus resection >1/3), untreated cruciate or collateral ligament laxity, untreated varus/valgus alignment >5°, obesity, inflammation, procedures in the respective knee (eg, microfracture or osteochondral autograft) less than 1 year ago, hyaluronan injection less than 6 months ago, and corticosteroid injection less than 3 months ago (Appendix). Randomization was performed using an Internet-based computer software assigning by chance patients to 1 of the 2 study groups in order to ensure consistency of observation.[17]

The study was performed in concordance with the Medical Association's professional code of conduct and with the Declaration of Helsinki in the version of 1996[18] and according to the German Data Protection Act of 1990.[15] Before the start of the investigation, written informed consent was obtained. The legal requirements concerning confidential medical communication were met. At any time, the patients had the right to withdraw consent without giving reasons—of course without disadvantages regarding further medical treatment. The medical therapy of the patients during hospitalization was performed independently of this research project according to standardized principles of the department of sports medicine and was not influenced or changed by this investigation. Patient data of the participants were stored exclusively in the Orthopedic University Hospital according to current data protection laws. Third persons were not provided insight into source data. Any patient had and still has the right to have his anonymized data deleted.

In Vitro Cultivation of Chondrocytes

For the cultivation of chondrocytes, approximately 250 mg of cartilage was removed from a healthy, less weightbearing area of the lateral aspect of the lateral femoral condyle during arthroscopy.

Blood sampling was performed during anesthesia. An amount of 40 mL blood serum corresponding to a sample of 90 mL whole blood was required. Cultivation of chondrocytes and preparation of m-ACI (BioSeed-C [BioTissue Technologies]) and ACI-P was performed by BioTissue Technologies in Freiburg, Germany. Chondrocytes were harvested enzymatically and expanded using autologous cell culture conditions. For m-ACI, 20 million autologous chondrocytes per BioSeed-C (20 × 30 × 2 mm) were embedded in fibrin (Tissucol Duo, Baxter AG, Unterschleissheim, Germany) and combined with the rectangular resorbable scaffold made of polyglactin 910 and poly-p-dioxanon. The chondrocytes were seeded on the scaffold 3 to 6 days before transplantation. For ACI-P, 15 million chondrocytes/500 μL were resuspended, filled into vials and shipped to the operation room. The amount of cells applied, of course, is dependent on defect size and volume of the defect cavity. Thus, for a hypothetical defect of 2 × 3 cm with a cartilage thickness of 2 mm, a volume of 1200 mm^3 (20 × 30 × 2 mm) is needed. A volume of 1200 mm^3 corresponds to 36 million cells (ACI-P). For the same hypothetical defect, 1 BioSeed-C (20 × 30 × 2 mm) with 20 million cells is sufficient (m-ACI). These cell counts correspond to the standard specifications for ACI-P and m-ACI, respectively.[4,26]

Surgical Procedures

After the chondrocytes were cultivated for 3 to 4 weeks, the patients were hospitalized a second time. Depending on lesion location, a medial or lateral parapatellar skin incision was made. After exposure of the lesion, the bottom of the defect was carefully debrided to normal surrounding articular cartilage. Care was taken not to penetrate the subchondral bone.

In the ACI-P procedure, initially a periosteal flap was removed from the anteromedial tibia surface. Subsequently, the periosteal flap was sutured cambium layer down (resorbable suture material 5-0/6-0) to the healthy cartilage rims and sealed with fibrin glue. Physiologic saline was used to test whether the cover was sealed appropriately. The chondrocyte-containing suspension was injected and the periosteal cover was closed. Finally, a lavage was performed, drainage inserted, and the wound closed.

In the m-ACI procedure, initially the defect size was measured, and subsequently, the chondrocyte-containing fleece tailored correspondingly. BioSeed-C was fixed in each corner with 2-0 Vicryl sutures (Ethicon, Somerville, New Jersey) secured by 3-fold knots. In each corner of the defect, Kirschner wires were drilled using the inside-out technique. The Vicryl sutures, attached to the knots, were pulled through the femoral bone using the Kirschner wires, and the knots were guided into the subchondral bone (Figure 1). The knots serve as bone anchors, securely fixing the graft. Lavage, drainage insertion, and wound closure followed. The postoperative rehabilitation program was the same for both patient groups. The patients were hospitalized for about 7 days. Starting the day after surgery, patients were subjected to continuous passive motion for 6 weeks. For 6 weeks, only partial weightbearing was allowed (sole contact). For 2 weeks, range of motion was restricted to 90° of flexion; afterward, full range of motion within pain tolerance was permitted. Sports were allowed as early as 12 weeks after ACI.

Magnetic Resonance Imaging Evaluation

Magnetic resonance imaging was performed using a 1-T unit (Gyroscan intera, Philips Medical Systems, Best, the

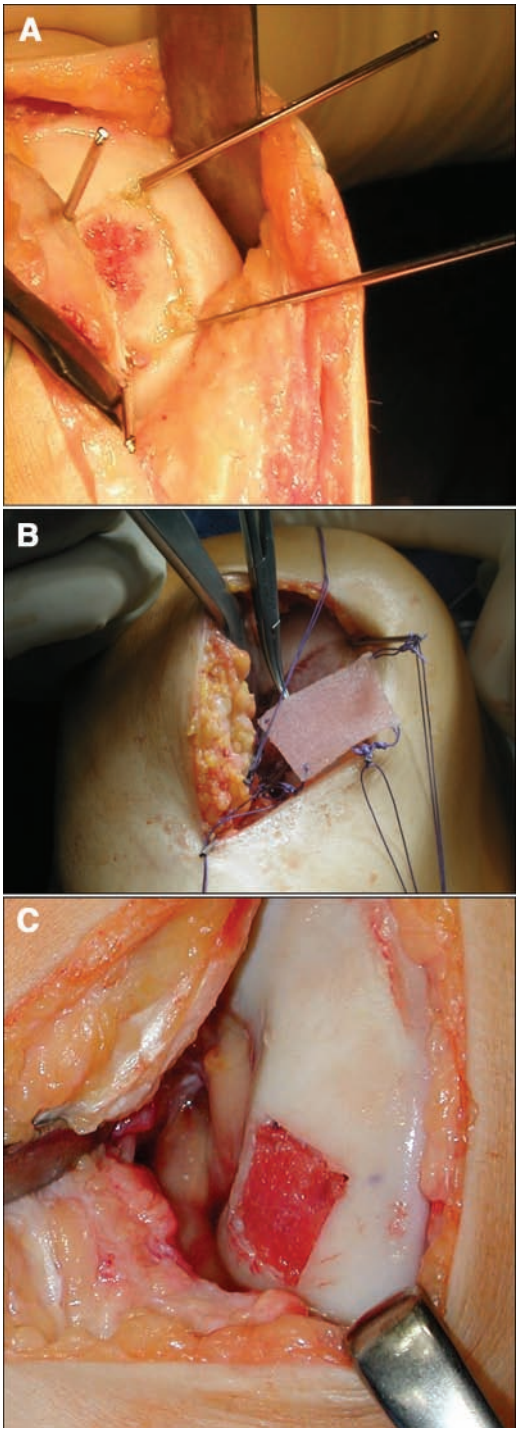

Figure 1. A, insertion of Kirschner wires at the edges of the cartilage defect. B, anchor knots attached at the edges of the BioSeed-C fleece are pulled in via the Kirschner wires (transosseous fixation). C, whole cartilage defect covered by BioSeed-C fleece.

Netherlands) with the use of a phased-array dedicated knee coil. Imaging included a coronal proton density (PD)-weighted spin-echo sequence (repetition time [TR]/echo time [TE], 2550/13.5), a coronal PD-weighted, fat-suppressed spin-echo sequence (TR/TE, 2550/13.5), a sagittal PD-weighted spin-echo sequence (TR/TE, 1500/13), a sagittal T1-weighted spin-echo sequence (TR/TE, 460/13), a transversal PD-weighted, fat-suppressed spin-echo sequence (TR/TE, 1500/12), and a coronal or transversal fat-suppressed gradient-echo sequence, (TR/TE, 13/60) with flip angle of 40°. All sequences were performed with a slice thickness of 3.0 mm and a field of view of 160. The gradient-echo sequence was optimized for cartilage imaging and was orientated tangential to the site of the graft, transverse for lesions of the trochlea and patellar lesions, and coronal for lesions of the condyle. An independent musculoskeletal radiologist blinded to the treatment groups assessed the postoperative MRI scans at 6, 12, and 24 months and the MOCART score (Magnetic Resonance Observation of Cartilage Repair Tissue)[32,33] was applied. This score has been developed for the evaluation of the regeneration of cartilage defects after therapy. The initial MRI scan was performed for the purpose of documentation.

Outcome Parameters

The primary outcome parameter (confirmatory analysis) was the postoperative change in subjective knee function as assessed by the International Knee Documentation Committee (IKDC) score[19] at 12 months. In addition, the postoperative change of IKDC at 3, 6, 12, and 24 months was analyzed in an exploratory fashion.

Secondary outcome parameters (exploratory analysis) were postoperative changes in health-related quality of life (Short Form 36 Health Survey [SF-36]),[8] knee functionality (Lysholm and Gillquist Score),[29] and physical activity (Tegner Activity Score [TAS])[42] at 3, 6, 12, and 24 months. Magnetic resonance imaging was performed to evaluate cartilage status at 6, 12, and 24 months.

Adverse events were recorded to assess safety.

Data Management

Written queries or queries by phone were performed for completeness of the data. Implausible data were corrected. Data were entered into the relational database Microsoft Access 2007 (Microsoft Corporation, Redmond, Washington), converted into SAS, Version 9.13 (SAS Institute, Cary, North Carolina), and subjected to further computer-assisted plausibility checks. Necessary admissible corrections (eg, an implausible date of birth) were made. Datasets were subsequently prepared for statistical analysis according to the instructions described in the original articles and/or handbooks of the standardized questionnaires including, for example, handling of missing data, recoding, and calculation of sum scores.[8,19,25,29,32,33,41,42]

Statistical Analysis

All statistical analyses were performed by using SAS Version 9.13 statistical software. The biostatistician who performed the analysis was not blinded to the treatment groups.

Descriptive Analysis. A descriptive analysis of the demographic variables was performed to investigate whether the study groups were homogeneous. Continuous variables were presented by mean and standard deviation, and qualitative variables by relative frequencies. Also, primary and secondary outcome parameters underwent descriptive analysis in order to compare baseline values and to quantify treatment effects.

Confirmatory Statistics. Confirmatory statistics were limited to the primary outcome parameter. Sample-size estimation was based on a 2-tailed test problem for the main outcome parameter. The α error and the power were set to 5% and 80%, respectively. The computer-assisted sample-size estimation was performed using an Internet-based computer software for 2-sample Student t test.[16] Henderson et al[13] reported an average postoperative change of IKDC score of +14.3 points after 12 months, without mentioning a standard deviation. Therefore, a standard deviation of 3.5 points was estimated from the reported data. Based on the data of Henderson et al and the hypothesis of the principal investigator, a difference between the means of 5 points with a standard deviation of 3.5 points was estimated. Accordingly, a minimal sample size of 8 patients per group was calculated. The sample size was adjusted to n = 9 per group considering that, depending on the distribution of values, the nonparametric 2-sample test by Mann and Whitney (Wilcoxon-Mann-Whitney test) has to be used. In this case, the sample size per group with a standard deviation of 3.5 points should be increased by the factor $\pi/3 = 1.05$. In addition, sample size was increased to at least 10 per group because of possible preliminary dropouts. When using the nonparametric 2-sample test (Wilcoxon-Mann-Whitney test) the Hodges-Lehmann estimator $\hat{\delta}$ for the difference between the groups $\hat{\delta} = \mu_2 - \mu_1$ with 95% confidence intervals was calculated by using the SAS macro tsp.sas.[6]

Exploratory Statistics. The secondary outcome parameters were analyzed using nonparametric tests without α -adjustment. The Hodges-Lehmann estimator $\hat{\delta}$ for the difference between the groups was again calculated by using the SAS macro tsp.sas. For analyzing changes over time within groups (eg, at ACI to 12 months postoperatively), the Wilcoxon test for paired samples was used. For analyzing the treatment and further influencing effects as well as interactions over the whole postoperative course of 24 months, mixed models developed for nonparametric analysis of longitudinal data in factorial experiments were calculated by using the SAS macro F1_LD_F1.sas.[6] For analyzing correlations between outcome parameters or between outcome parameters and influencing factors, Spearman correlation coefficients r were calculated.

RESULTS

Description of the Study Population

The first patient underwent surgery on March 11, 2004 and the last on June 19, 2006. By June 30, 2008, in total 21 patients (16 males, 5 females) had been treated with either m-ACI or ACI-P and had completed a follow-up of 24 months. A detailed description of the study population is given in the Appendix. There were 6 men and 5 women (mean age ± standard deviation: 29.1 ± 7.5 years) in the m-ACI group and 10 men with a mean age of 29.5 ± 11.0 years in the ACI-P group. Mean weight was 76.0 ± 14.2 kg in the m-ACI group and 81.9 ± 11.6 kg in the ACI-P group; mean height was 175.5 ± 11.1 cm in the m-ACI group and 181.0 ± 7.5 cm in the ACI-P group. Ten patients (47.6%) were of a healthy weight, and 9 patients (42.9%) were overweight (body mass index [BMI] >25 kg/m^2 and ≤30 kg/m^2). Only 1 patient (4.8%) with a BMI of 30.4 kg/m^2 was borderline obese and 1 patient (4.8%) had a BMI of only 19.6 kg/m^2. Cartilage defect size was on average 4.3 ± 1.1 cm^2 in the m-ACI group and 4.1 ± 0.9 cm^2 in the ACI-P group. All defects were rated grade IV. Within this RCT, 9 right and 12 left knees were treated. Most of the cartilage defects (18 of 21 [85.7%]) were localized at the medial femoral condyle. The patients had symptoms before ACI on average for 24.2 ± 26.8 months in the m-ACI and 32.7 ± 34.9 months in the ACI-P group. Before ACI, the patients had undergone on average 2.1 ± 1.2 surgical procedures in the respective knee in the m-ACI group and 1.9 ± 0.7 in the ACI-P group. To perform an m-ACI procedure, incision to suture time took on average 86.7 ± 9.5 minutes and an ACI-P procedure took 112.5 ± 19.3 minutes. A detailed description of previous and concomitant surgical procedures is given in the Appendix.

Confirmatory Statistical Analysis

The IKDC score increased in the m-ACI group from 51.1 ± 22.8 at baseline by +20.9 ± 20.9 points to 72.0 ± 22.7 at 12 months. Regarding the ACI-P group, the IKDC score increased from 52.0 ± 13.5 at baseline by +24.6 ± 19.3 points to 76.6 ± 18.9 at 12 months. There was no difference between the 2 ACI methods with regard to the postoperative change in subjective knee function 12 months after ACI (P = .5573); the Hodges-Lehmann estimator $\hat{\delta}$ for the difference between the groups was 3.5 points (95% confidence interval –14.4, 20.7) (Table 1).

Exploratory Statistical Analysis

International Knee Documentation Committee Score. The exploratory analysis of IKDC changes over time (at ACI to 12 months postoperative) within the study groups showed a significant improvement of knee function in both study groups (m-ACI, P = .0039; ACI-P, P = .0039) compared with the preoperative situation.

Two years after ACI, the m-ACI patients obtained an average IKDC score of 70.1 ± 28.6 and the ACI-P patients, 77.1 ± 22.7. There was no difference between the 2 ACI methods with regard to the postoperative change in subjective knee function 24 months after ACI (P = .4994) (Table 1). The exploratory analysis of IKDC changes over time (at ACI to 24 months postoperative) within the study groups showed a significant improvement of the knee function only in the ACI-P group

TABLE 1
Outcome Measures: Postoperative Change of IKDC Score, Lysholm and Gillquist Score, and Tegner Activity Score 12 and 24 Months After ACI (N = 21)[a]

Outcome Measure	Postoperative Change 12 Months After ACI (Mean ± Standard Deviation)	Hodges-Lehmann Estimator $\hat{\delta}$ (and 95% Confidence Interval) for the Difference Between the Groups 12 Months After ACI	m-ACI vs ACI-P, Postoperative Change 12 Months After ACI, Wilcoxon-Mann-Whitney Test, *P* Value	Postoperative Change 24 Months After ACI (Mean ± Standard Deviation)	Hodges-Lehmann Estimator δ (and 95% Confidence Interval) for the Difference Between the Groups 24 Months After ACI	m-ACI vs ACI-P, Postoperative Change 24 Months After ACI, Wilcoxon-Mann-Whitney test, *P* Value
IKDC score	m-ACI: +20.9 ± 20.9 ACI-P: +24.6 ± 19.3	3.5 (–14.4, 20.7)	.5573	m-ACI: +19.0 ± 26.8 ACI-P: +25.2 ± 23.2	2.9 (–14.9, 29.9)	.4994
Lysholm and Gillquist score	m-ACI: +4.9 ± 19.0 ACI-P: +25.0 ± 22.8	22.5 (1.0, 41.0)	.0449[b]	m-ACI: +1.2 ± 22.3 ACI-P: +22.7 ± 25.9	24.0 (1.0, 48.0)	.0487[b]
TAS	m-ACI: +0.1 ± 2.1 ACI-P: +0.9 ± 2.5	1.0 (–1.0, 3.0)	.4063	m-ACI: +0.6 ± 2.7 ACI-P: +1.7 ± 2.0	1.0 (–0.5, 3.0)	.1043

[a]IKDC, International Knee Documentation Committee; ACI, autologous chondrocyte implantation; m-ACI, matrix-associated ACI; ACI-P, ACI using the original periosteum-cover technique; TAS, Tegner Activity Score.
[b]Significant; $P < .05$.

(m-ACI, $P = .0635$; ACI-P, $P = .0098$) compared with the preoperative situation.

To investigate the influence of treatment method on the IKDC course over the whole observation period, in a mixed model, all points in time were included (time 0, and 3, 6, 12, and 24 months). Neither the method of surgery ($P = .7571$) nor the interaction of method and time ($P = .5751$) showed a significant effect on the development of the knee function (IKDC score) over time. There was only a significant effect of time ($P < .001$). In addition, age was found to have an independent significant effect on the development of knee function (IKDC score), with better treatment results being found in younger patients (extended mixed model, $P = .0001$). The IKDC score was inversely correlated to age; Spearman correlation coefficients $r = –.52$ at 12 months and $r = –.49$ at 24 months.

The IKDC results of men and women within the m-ACI group were equivalent, and so were the IKDC results in both study groups when considering only men (descriptive analysis, data not shown).

Short Form 36 Health Survey (SF-36). The patients' health after m-ACI and ACI-P was assessed by 8 categories: physical functioning (PF), physical role (PR), physical pains (PA), global health (GA), vitality (VI), social functioning (SF), emotional role (ER), and psychological well-being (PS).

There were no significant differences between the study groups regarding the postoperative change of the 8 categories at 12 and 24 months (data not shown).

Within the m-ACI group, significant changes over time compared with time 0 were found for PR ($P = .0156$) and PA ($P = .0391$) at 12 months, and PF ($P = .0020$) and PR ($P = .0156$) at 24 months.

Within the ACI-P group, significant changes over time compared with time 0 were observed for PF ($P = .0059$) at 12 months and PF ($P = .0156$) at 24 months.

Lysholm and Gillquist Score. The Lysholm and Gillquist Score increased in the m-ACI group from 71.4 ± 23.8 at time 0 to 76.3 ± 27.5 at 12 months, and in the ACI-P group from 61.3 ± 14.3 at time 0 to 86.3 ± 17.0 at 12 months. There was a significant difference between the study groups regarding the postoperative change measured 1 year after surgery favoring the ACI-P group ($P = .0449$) (Table 1). The analysis of Lysholm and Gillquist score changes over time (at ACI to 12 months postoperative) within the study groups showed a significant improvement of knee function only in the ACI-P group (m-ACI, $P = .4639$; ACI-P, $P = .0137$) compared with the preoperative situation.

Two years after ACI, m-ACI patients obtained an average Lysholm and Gillquist score of 72.5 ± 28.0 and ACI-P patients, 84.0 ± 21.9. There was also a significant difference between the study groups regarding the postoperative change measured 2 years after surgery favoring the ACI-P group ($P = .0487$) (Table 1). The analysis of Lysholm and Gillquist score changes over time (at ACI to 24 months postoperative) within the study groups again showed a significant improvement of knee function only in the ACI-P group (m-ACI, $P = .9150$; ACI-P, $P = .0273$) compared with the preoperative situation.

Analysis of the change in the Lysholm and Gillquist score in a mixed model including all points in time showed that there was no significant influence of the method of treatment ($P = .8712$) or the interaction between method of treatment and time ($P = .0638$), while time had a significant effect ($P < .0001$). Again, there was an independent effect of age (extended model, $P = .0013$).

Tegner Activity Score. The TAS decreased from 4.1 ± 2.8 in the m-ACI group and 3.7 ± 1.9 in the ACI-P group at time 0 within the first 3 months after ACI and increased again during the following 9 months, reaching at 12 months the preoperative level in the m-ACI group (4.2 ± 2.0) and a higher level, compared with baseline, in the ACI-P group (4.6 ± 2.0). There was no difference in the postoperative change of TAS between the 2 ACI methods at 12 months ($P = .4063$) (Table 1). The analysis of TAS changes over time (at ACI to 12 months postoperative) within the study groups did not show a significant improvement in both groups (m-ACI, $P = .9141$; ACI-P, $P = .2500$) compared with the preoperative situation.

Two years after ACI, m-ACI patients reached an average TAS of 4.7 ± 2.9 and ACI-P patients, 5.3 ± 1.9. There was no difference in the postoperative change of TAS between the 2 ACI methods at 24 months ($P = .1043$) (Table 1). The analysis of TAS changes over time (at ACI to 24 months postoperative) within the study groups did not show a significant improvement in both groups (m-ACI, $P = .7832$; ACI-P, $P = .0625$) compared with the preoperative situation.

When considering all points in time in a mixed model, a significant time effect ($P < .0001$) was evident, but neither an influence of method ($P = .6659$) nor of interaction between method and time ($P = .3369$). Again, there was an independent effect of age (extended model, $P = .0277$).

Magnetic Resonance Imaging and MOCART Score. A total of 17 of the 21 patients completed the MRI examinations at 6 and 12 months. Regarding the item "defect repair and filling" at 6 months, complete defect filling was more frequent in the m-ACI group (m-ACI, 4 of 8; ACI-P, 1 of 9 patients), hypertrophy was more frequent in the ACI-P group (m-ACI, 1 of 8; ACI-P, 7 of 9 patients), and an incomplete defect repair more frequent in the m-ACI group (m-ACI, 3 of 8; ACI-P, 1 of 9 patients). Only in 2 patients of the m-ACI group was a complete integration to the border zone observed at 6 months (m-ACI, 2 of 8; ACI-P, 0 of 9 patients) and in 3 patients (all of them m-ACI) at 12 months. Regarding the item "surface of the repair tissue," similar results were obtained in both study groups up to 12 months. As to the item "structure of the repair tissue," in the m-ACI group at 6 months in 4 patients, a homogeneous repair tissue was evident, whereas in the ACI-P group, only 1 patient had homogeneous repair tissue at 12 months. Isointensity was seen earlier (at 6 months) in the m-ACI group, when MRI was performed in the PD fat-suppressed mode, but not in the PD or 3D gradient-echo fat-suppressed mode. At 6 months, in 4 patients of the m-ACI group and 2 patients of the ACI-P group, an intact subchondral lamina was observed, and at 12 months in 7 patients (m-ACI) and 5 patients (ACI-P), respectively. Regarding the item "subchondral bone," there were obvious differences between the study groups. An intact subchondral bone was seen earlier and more frequently in the m-ACI group, and granulations, tissue cysts, or sclerosis were more frequent in the ACI-P group.

In both study groups, adhesions did not play an important role; at 12 months, adhesions were no longer found in the m-ACI group. Effusion turned out to be a rather frequent problem both at 6 and 12 months, and there were no obvious differences between the study groups.

Six months after surgery (n = 17), a significantly lower MOCART score was obtained in the m-ACI group (m-ACI, 7.0 ± 2.7; ACI-P, 10.3 ± 1.6; $P = .0123$), corresponding to more positive (more normal) MRI diagnostic findings; 12 months (n = 17) as well as 24 months (n = 11) after ACI, the differences between the study groups were not significant (Table 2).

Correlation matrices for 6, 12, and 24 months showed that IKDC score, Lysholm and Gillquist score, and TAS were correlated among each other but none of them with the MOCART score (data not shown). Regarding SF-36, the MOCART score at 24 months proved to be inversely correlated with physical role (PF) ($r = –.68$) as well as social functioning (SF) ($r = –.71$).

Adverse Events

In 1 patient of the ACI-P group, transplant delamination was observed by MRI 6 months after surgery. One female patient of the m-ACI group developed an osteochondral lesion. It is not clear whether it was an osteochondritis dissecans lesion or maybe a partial ossification and delamination of the graft that was mistaken for an osteochondritis dissecans lesion. She complained of persisting pain. Effusion was a rather frequent adverse event. At 6 months, effusion was observed in 11 patients (m-ACI, n = 5; ACI-P, n = 6) and at 12 months in 10 patients (m-ACI, n = 5; ACI-P, n = 5). Transplant hypertrophy occurred frequently and was more common in the ACI-P group. At 6 months, it was seen in 8 patients (1 m-ACI, 7 ACI-P) and at 12 months in 9 patients (3 m-ACI, 6 ACI-P). Transplant hypertrophy, however, was not associated with symptoms. In 1 patient of the ACI-P group, the development of an osseous spur was observed 12 months after surgery.

Four patients underwent revision arthroscopy (m-ACI, 3 of 11 patients vs ACI-P, 1 of 10; $P = .5865$, Fisher exact test). Revision arthroscopy was performed for patients who had persisting pain. More importance was attached to the clinical symptoms than to the MRI findings. Typical clinical symptoms were foreign body sensation, frequent swelling, and pressure pain over the joint space. In one case a pure "second-look" arthroscopy was performed concomitant to a planned metal plate removal. Three of the 4 patients who underwent revision arthroscopy were symptom-free afterward.

One patient in the m-ACI group had persistent problems and could not return to work.

There were no cases of infection, allergic, toxic, or autoimmune reaction or malignant transformation.

DISCUSSION

Our RCT documents that the regenerative cartilage repair techniques ACI-P and m-ACI, which use autologous chondrocytes embedded in fibrin and in a polyglycolic acid-based scaffold, show equivalent efficacy at 12 and 24 months after surgery with respect to the patients' knee function as assessed by the IKDC score. Comparable results were also found for the SF-36.

With respect to the Lysholm and Gillquist score, a secondary outcome parameter, we found a significant difference of the postoperative improvement between the study groups favoring the ACI-P patients at 12 and 24 months. However, in our study, these significant differences observed for the Lysholm and Gillquist score could neither be confirmed by the results obtained with the primary outcome measure, the IKDC subjective knee form, nor for the secondary outcome measures, the TAS and SF-36 subscales.

To our knowledge, so far there is no study that showed superiority of m-ACI compared with lower-generation ACI.

TABLE 2
Magnetic Resonance Observation of Cartilage Repair (MOCART) Score 6, 12, and 24 Months After ACI[a]

Time After ACI	6 Months (n = 17)	12 Months (n = 17)	24 Months (n = 11)
MOCART score (mean ± standard deviation)	m-ACI: 7.0 ± 2.7 ACI-P: 10.3 ± 1.6	m-ACI: 6.3 ± 3.5 ACI-P: 8.4 ± 2.2	m-ACI: 6.3 ± 3.0 ACI-P: 6.8 ± 4.7
Hodges-Lehmann estimator δ (and 95% confidence interval) for the difference between the groups	3.0 (1.0, 5.6)	2.4 (–1.0, 5.0)	1.0 (–5.0, 6.0)
m-ACI vs ACI-P, MOCART score, Wilcoxon-Mann-Whitney test, P value	.0123[b]	.2065	.6926

[a]A lower MOCART score corresponds to more normal MRI diagnostic findings. ACI, autologous chondrocyte implantation; m-ACI, matrix-associated ACI; ACI-P, ACI using the original periosteum-cover technique.
[b]Significant; $P < .05$.

The results found for the main outcome measure are consistent with the findings of Manfredini et al,[30] who compared ACI-P and m-ACI based on a hyaluronan scaffold in an observational case report study. In this study, the original ACI and the third-generation m-ACI procedure showed comparable short-term and midterm results as assessed by the International Cartilage Repair Society scores and MRI.

Bartlett et al,[2] for instance, performed a prospective, randomized study comparing ACI-C (ACI covered with a collagen sheet) and m-ACI for the treatment of symptomatic chondral defects of the knee in 91 patients. Forty-four patients received ACI-C and 47, m-ACI grafts; a second-look arthroscopy performed 1 year after transplantation revealed a good to excellent International Cartilage Repair Society score in 79.2% of patients in the ACI-C group and 66.6% in the m-ACI group. Hyaline-like cartilage or hyaline-like cartilage with parts of fibrous cartilage was found in 43.9% of the biopsies in the ACI-C and 36.4% in the m-ACI transplants 1 year postoperatively. The incidence of hypertrophy was 9% (4 of 44) in the ACI-C group and 6% (3 of 47) in the m-ACI group. The authors concluded that the clinical, arthroscopic, and histologic results for ACI-C and m-ACI were very similar.

Recently, in a comparative study with a 5-year follow-up, it has been shown that third-generation, scaffold-based ACI significantly improved the clinical outcome compared with microfracture.[24] Interestingly, in comparative studies using the original ACI procedure and microfracture treatment, no difference with respect to the clinical outcome was evident after 2 and 5 years.[22,23] Similar results were reported recently by Saris et al,[38] who compared ACI and microfracture for cartilage repair in a cohort of 118 patients; 1 year after surgery, both treatment regimens showed similar clinical outcomes, but from the histologic point of view, the repair tissue was better in the ACI group compared with microfracture. In addition, 36-month clinical data of this study even show a superiority of this technique compared with microfracture (van Asche et al, unpublished data presented at the 8th World Congress of the International Cartilage Repair Society in Miami Florida, May, 2009). However, this technique really differs from conventional ACI because an autologous cartilage cell therapy product was implanted that has been optimized for its biological potency to form stable cartilage tissue in vivo.

A main factor influencing the clinical outcome seems to be the patient's age, which was an independent influencing factor with respect to IKDC score, Lysholm and Gillquist score, and TAS. This observation is consistent with Krishnan et al,[27] who found that younger patients benefit more from ACI-C compared with older patients; McNickle et al,[34] who found an association between clinical outcome after ACI (Lysholm and Gillquist score) and age favoring younger patients; and Marcacci et al,[31] who observed better clinical results after m-ACI in young athletic patients. Although there seems to be a trend toward inferior ACI results in older patients, the sample size in this study is too small to provide age-based recommendations for ACI.

Although the clinical outcome parameters, except for the Lysholm and Gillquist score, were comparable between the ACI-P and m-ACI groups, significant differences were observed regarding the MRI findings. A significantly lower MOCART score[32] was obtained in the m-ACI group 6 months after ACI. Particularly the items defect repair and filling, integration into the border zone, structure of repair tissue, as well as subchondral bone were different in the 2 study groups, with transplant hypertrophy, granulations, cysts, and sclerosis being more frequent in the ACI-P group and complete defect filling, complete integration into the border zone, homogeneous repair tissue, as well as an intact subchondral bone in the m-ACI group. However, during further postoperative development, MRI showed similar results in both study groups. The m-ACI patients using polyglycolic acid–based scaffolds showed earlier defect filling and transplant integration compared with the ACI-P group (although 3 patients of the m-ACI group still had an incomplete defect filling 6 months after ACI). However, it still has to be elucidated whether better repair tissue is clinically relevant in the long term.

In the ACI-P group, revision surgery was indicated in 1 of 10 cases (10%), while in the m-ACI group, second-look arthroscopies were performed in 4 of 11 cases (36.4%). In the patient (m-ACI) who had high tibial osteotomy, a second-look arthroscopy was performed concomitant with plate removal and would not have been conducted as a sole procedure. Considering this fact, the revision rate of the m-ACI group was 3 of 11 (27.3%). For the ACI-P group, the revision rate is in concordance with other studies

reporting rates of revision surgery between 0% and 25%.[28,35] Actually, we do not have a plausible explanation for the observed difference. In a study on complications after ACI, Niemeyer et al[36] evaluated 309 patients at a mean follow-up of 4.5 years. Revision arthroscopy rates of 14 of 52 (26.9%) for ACI-P, 26 of 215 (12.1%) for ACI-C (Chondro-Guide–covered), and 12 of 82 patients (14.6%) for m-ACI (BioSeed-C) were reported. The overall revision rate after ACI was increased for ACI-P ($P = .008$). Thus, a bigger sample size may be needed to assess revision rates.

A higher incidence of transplant hypertrophy for ACI-P is well known.[1,2,4,12,36] In particular, the technical advantage of m-ACI is the absence of any cover material like a periosteal flap that may cause transplant delamination and periosteal hypertrophy. In addition, stable scaffolds like polymer-based textile materials allow for secure fixation of the grafts by transosseous or cartilage suture as well as pin fixation.[9,21] Because in m-ACI the chondrocytes are embedded and fixed in scaffolds, third-generation ACI may even be used for the treatment of cartilage defects without an intact defect shoulder, which often occurs in degenerative defects. In addition, stable scaffolds allow for the arthroscopic implantation of the graft, thus reducing the morbidity associated with open procedures. Recently, Kreuz et al[26] showed, in an observational case report study with a 4-year follow-up, that m-ACI based on polyglycolic acid scaffolds significantly improved the outcome of patients with focal degenerative cartilage defects. The promising use of third-generation ACI for the treatment of osteoarthritic knees has also been shown in the short term using hyaluronan-based scaffolds. Second-look biopsies documented that cartilage regeneration occurred in 10 of 23 patients 1 year after surgery and that osteoarthritis did not inhibit the regeneration progress.[14] Therefore, third-generation ACI shows technical advantages that may open new vistas for the treatment of degenerative defects without intact cartilage rims. In addition, m-ACI allows for significantly shorter operation times (m-ACI, 86.7 ± 9.5 vs ACI-P, 112.5 ± 19.3; $P = .0135$, Wilcoxon-Mann-Whitney test) as it is no longer necessary to remove a periosteal flap to cover the cartilage defect. This fact may substantially reduce the treatment costs and make this expensive new technique affordable.

Limitations to this study are the small sample size and the unbalanced gender ratio. From the knowledge we now have on the clinical outcome of ACI techniques, the a prior standard deviation of 3.5 for the postoperative change of IKDC scores was an underestimation. Between 2003 and 2009, a number of studies on ACI have been published that support this point. At the time this RCT was planned, knowledge of this new technique was insufficient to accurately estimate the sample size. Indeed, this RCT is underpowered. Consequently, this study presents preliminary data in a randomized fashion for a new procedure.

Randomization was not stratified by gender, so that by chance all women got m-ACI; this is a further weakness of this RCT. Although, we did not find a gender effect regarding the main outcome measure within the m-ACI group, this result does not allow for conclusions because of the small sample size and because all women were in the m-ACI group. Nevertheless, the lack of a generalized effect on ACI results in this study is consistent with the findings of other researchers.[27] Similarly, all lateral condyle lesions were in the m-ACI group, so we could not assess the effect of lesion location.

The results of this investigation should be tested by performing an RCT stratified by gender and age with a larger sample size and a longer follow-up (>24 months).

CONCLUSION

This RCT confirmed the efficacy of ACI and m-ACI based on polyglycolic acid scaffolds in the treatment of cartilage defects in the femoral condyle. Superiority of either the m-ACI or the ACI-P technique with respect to the primary outcome measure (ie, IKDC score) was not evident, nor for the secondary outcome measures SF-36 and TAS. But a significant difference was found for the secondary outcome measure of postoperative change in Lysholm and Gillquist score 12 and 24 months after ACI. Age might be the main factor influencing the clinical outcome.

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