Is the Transplant Quality at the Time of Surgery Adequate for Matrix-guided Autologous Cartilage Transplantation? A Pilot Study

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Abstract

Background Matrix-guided autologous chondrocyte transplantation (MACT) has been proposed as an option for treating large full-thickness cartilage defects. However, little is known about the chondrogenic potential of transplants for MACT at the time of implantation, although cell quality and chondrogenic differentiation of the implants are crucial for restoration of function after MACT.

Questions/purposes We therefore asked: (1) Do MACT implants allow deposition of extracellular cartilage matrix in an in vitro culture model? (2) Are these implants associated with improved knee function 1 year after MACT in large cartilage defects?

Each author certifies that he or she, or a member of his or her immediate family, has no funding or commercial associations (eg, consultancies, stock ownership, equity interest, patent/licensing arrangements, etc) that might pose a conflict of interest in connection with the submitted article.

All ICMJE Conflict of Interest Forms for authors and *Clinical Orthopaedics and Related Research* editors and board members are on file with the publication and can be viewed on request. Each author certifies that his or her institution approved the human protocol for this investigation, that all investigations were conducted in conformity with ethical principles of research, and that informed consent for participation in the study was obtained. This work was performed at the University of Regensburg, Regensburg, Germany, and the Sporthopaedicum Regensburg, Regensburg, Germany.

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Methods We retrospectively reviewed all 125 patients with large localized cartilage defects (mean defect size 5 cm²) of the knee who were treated with MACT from 2005 to 2010. The mean age was 31 years (range, 16–53 years). Portions of the cell-matrix constructs (n = 50) that were not implanted in the cartilage defects were further cultured and tested for their potential to form articular cartilage. Knee function of all patients was analyzed preoperatively, 3 months, and 1 year postoperatively with the International Knee Documentation Committee (IKDC) score.

Results In vitro assessment of the cell-matrix implants showed chondrogenic differentiation with positive staining for glycosaminoglycans and collagen II in all cultures. Enzyme-linked immunosorbent assay showed an increase of collagen II production. We observed an improvement in median IKDC score from 41 to 67 points at last followup.

Conclusions Cartilage extracellular matrix deposition shows adequate implant quality for MACT at the time of implantation and justifies the use for treatment of large cartilage defects.

Level of Evidence Level IV, therapeutic study. See Guidelines for Authors for a complete description of levels of evidence.

Introduction

Brittberg et al. [8] introduced the technique of autologous chondrocyte transplantation (ACT) in 1994. Conventional

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ACT involves the harvesting of osteochondral plugs, isolation and cultivation of chondrocytes from the plugs, and injection of a chondrocyte cell suspension into the defect site after covering with a periosteal flap. In cartilage defects larger than 3 cm², conventional ACT reportedly is associated with functional improvement at 2 to 10 years [30, 31] and superior function compared with microfracture after 5 years [7]. However, hypertrophy of the periosteum with subsequent revision reportedly occurs in 26% of patients [32] with a risk of transplant failure in 5% to 25% of patients [11].

Other treatment options like microfracturing (MF) or osteochondral transplantation are associated with disadvantages, especially for treatment of large cartilage defects (ie, > 4 cm²) compared with matrix-guided autologous chondrocyte transplantation (MACT) [6, 12]. In smaller defects (< 2 cm²), MF reportedly reduces pain, improves mobility, and allows return to sport [18]. However, the defects heal with fibrocartilage with inferior mechanical qualities [16] and over time active patients with larger defects experience reduced function [18, 25]. Intralesional osteophytes occur in 20% to 40% of patients 4 or 5 years after MF [9, 25, 27, 35].

The MACT was developed to enhance repair and provide more durable function. MACT involves harvesting of osteochondral plugs, isolating and cultivating chondrocytes, seeding of a matrix, and implanting the matrix (rather than a cell suspension) in the cartilage defect. Compared with conventional chondrocyte transplantation, MACT has the potential to reduce disadvantages such as periosteal hypertrophy and high revision rates [11, 23, 34]. In a randomized controlled prospective study of 60 patients with large (4–10 cm²) chondral defects, MACT was associated with higher Lysholm and Tegner scores at 6, 12, and 24 months compared with MF [5]. Reasons for superior clinical function after MACT compared with MF might be better defect filling with cartilage tissue [42] and the absence of intralesional osteophytes. Complete filling with well-differentiated tissue correlates with improved knee function [34]. On the other hand, incomplete defect filling with undifferentiated scar tissue is associated with a risk of persistent pain and lower function, particularly in larger chondral defects [14, 17, 21, 26]. Although the importance of a high quality of repair tissue for the patient's outcome after MACT is well described [27, 32, 34], the chondrogenic potential of the chondrocyte-matrix construct itself at the time of implantation is unclear.

We therefore determined whether (1) MACT implants allow deposition of extracellular cartilage matrix and (2) these implants are associated with improved knee function 1 year after MACT in large cartilage defects.

Patients and Methods

Between 2005 and 2010, we treated 125 patients with symptomatic large cartilage lesions of the knee with MACT. The indications for MACT were: defect size > 3 cm² and/or failure of other previous cartilage treatments. The contraindications were arthritis of the knee, rheumatoid arthritis, or infection of the knee (see also Schewe et al. [36]). During that same time, we treated 506 patients with cartilage defects of the knee of all sizes. Of the 125 patients, 75 were men and 50 were women. Mean age was 31 years (range, 16–53 years). The mean size of the defects was 5 cm² (range, 3–13 cm²). Fifty patients were lost to followup at ranges from 3 to 12 months or were excluded because of incomplete documentation. This left 75 of the 125 patients with a complete evaluation at 1 year postoperative.

The 75 patients had 79 cartilage lesions that were treated with MACT. The most common defect location was the medial condyle (n = 48). Other locations were the retro-patellar surface (n = 18), the lateral condyle (n = 8), the trochlea (n = 3), and the medial tibial plateau (n = 2). Thirty-five patients had previous operations of the knee like MF, ACL reconstruction, or meniscal treatment. Before operative treatment, the duration of symptoms in most cases was longer than 1 year.

Preoperative assessments included medical history, physical examination, radiographs of the knee in three planes, long leg standing radiographs, MRI of the knee, and the International Knee Documentation Committee (IKDC) scores [13]. On the radiographs we evaluated degenerative changes, comorbidities, and leg axis.

We performed arthroscopy to evaluate the chondral lesions. We judged a lesion suitable for MACT if it had the following characteristics: focal traumatic, osteochondritis dissecans, and focal degenerative grade 3 and 4 lesions according to the International Cartilage Repair Society classification [23]. If we deemed the lesion suitable for a MACT, we took two or three osteochondral plugs from the osteoarticular margin on the lateral wall of the notch of the knees.

The osteochondral plugs were sent to a certified laboratory (TETEC AG, B. Braun Aesculap AG, Reutlingen, Germany) for further preparation. The chondrocytes were isolated cultured and amplified over 3 weeks. Before transplantation, scaffolds were seeded with the cultured cells. The matrix has two layers: one layer is a porous, resorbable collagen sponge, which allows incorporation of the chondrocytes and provides a suitable milieu for cell adhesion and differentiation. A second layer, which is dense and not accessible for cell incorporation, is made from bovine pericardium. The pericardium gives stability

Fig. 1A–F MACT of a large cartilage defect located at the medial condyle of a right knee in a 33-year-old patient. First the defect area was prepared until the defect edges were stable (A). Then the implant was cut into the right size (B), placed in the defect, and fixed with sutures (C). For successful cartilage therapy, it is necessary to address all comorbidities. Therefore, the patient had a high tibial osteotomy to

correct the varus deformity (D). At this procedure 4 weeks after the MACT, the defect was evaluated arthroscopically. At this early stage of healing, a stable cell layer can be seen in the defect area (E). At the time of removal of the angular stable plate 1 year after MACT, the arthroscopic view of the medial condyle showed a completely filled defect with stable cartilage-like repair tissue (F).

to the cell-matrix constructs and allows proper intraoperative handling and fixation of the implants. After 3 weeks of cultivation, the cell-matrix constructs were sent to the hospital for implantation.

All operations were performed in the same setting by one experienced surgeon (PA). Through miniarthrotomy either on the medial or on the lateral side of the patella, the cartilage defects were exposed and unstable cartilage was completely removed with a curette to stable rims. The cell-matrix constructs were then placed in the defects and fixed with resorbable 6-0 sutures (Fig. 1). Then the joints were moved several times to ensure stable fixation of the transplants followed by suture of the joint capsule and of the skin. In 11 defects, additional transplantation of autologous bone grafting (cancellous bone and/or cortical bone block) underneath the MACT was necessary to fill the osseous defect. In some cases, the MACT was combined with one or more procedures like ACL reconstruction ($n = 18$), meniscal suture ($n = 15$), and osteotomy ($n = 17$).

After preparation of the implants according to the cartilage defect geometries, the remnants of the cell-matrix constructs

of 50 patients were further evaluated in vitro. In the remaining 25 cases the remnants were not large enough to perform further analysis. To assess cartilage differentiation, we cut the remnants of the constructs in cubical pieces ($4 \text{ mm} \times 4 \text{ mm}$) and cultured them under chondrogenic conditions for 1, 7, 14, or 21 days. Differentiation was induced by treatment with serum-free high-glucose DMEM (Gibco Invitrogen, Darmstadt, Germany) containing 100 nM dexamethasone (Sigma, Steinheim, Germany), 1% ITS 3 (insulin–transferrin–selenium) (Sigma), 200 μM L-ascorbic acid 2-phosphate (Sigma), 1% sodium pyruvate (solution) (Gibco Invitrogen), and 10 ng/mL human transforming growth factor $\beta 1$ (R&D Systems, Wiesbaden, Germany) [2–4]. Three to five samples from each time point were examined histologically, immunohistologically, and were assayed for chondrogenesis related to content of collagen type II. For the histological analysis, samples were fixed in 4% phosphate-buffered paraformaldehyde for 60 minutes, rinsed, and then infiltrated with increasing concentrations (10%–30%) of sucrose in phosphate buffer. The samples were then embedded in Tissue-Tek (Sakura, Zoeterwoude, The Netherlands), frozen in liquid

nitrogen, and cryosectioned at 10 to 12 μm with an HM 500 OM cryotome (Microm, Berlin, Germany). The metachromatic dye 1,9-dimethylmethylene blue (DMMB; Sigma) 0.1% in H_2O was used to detect the synthesized sulfated glycosaminoglycans (sGAG) [37]. sGAG content was measured by DMMB color change from blue to purple. Expression of collagen type II was evaluated on predigested sections (3 mg/mL pepsin [Sigma], citric/phosphate McIlvaine buffer [15 minutes]) by the immunoreaction with monoclonal primary mouse antihuman collagen type II antibodies (clone II-4C11; Calbiochem-Merck, Schwalbach, Germany) followed by biotin-conjugated polyclonal secondary goat antimouse IgG antibodies (Jackson, West Grove, PA, USA). The immunoreaction was visualized by the immunoperoxidase ABC technique (Vector, Burlingame, CA, USA) according to Hsu et al. [15] and DAB; 3,3'-diaminobenzidine (Sigma) 0.5 mg/mL, in phosphate buffer pH 7.4, containing 0.002% $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.04% $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ and 0.012% H_2O_2 [20].

Two examiners (JZ, RK), experienced in histological cartilage evaluation, analyzed all histological sections to assess chondrogenic differentiation and type II collagen. Differentiation was reliably judged by amount of metachromatic staining indicated by color change from blue to purple [2, 37] and positive collagen II immunostaining [26]. Both examiners agreed completely (interrater agreement kappa = 1) in assessing extracellular matrix deposition indicated by purple metachromatic staining and collagen II immunostaining.

In 25 of the 50 cases, we performed an enzyme-linked immunosorbent assay test for collagen II on remnants of the constructs. Samples of Novocart 3D (B. Braun Aesculap AG) cultured with chondrocytes were homogenized in 0.05 M acetic acid plus 0.5M NaCl (pH 2.9–3.0), digested with 10 mg/mL pepsin, and dissolved in 0.05 M acetic acid on the rotator for 48 hours at 4° C. The further steps of digestion, the preparation of standards, and the collagen type II estimation were performed as described in the Native Type II Collagen Detection Kit 6009 protocol (Chondrex, Redmond, WA, USA). The DNA concentration in collagen digests was assayed for using the Quant-iT dsDNA Assay Kit (Invitrogen, Eugene, OR, USA).

For cytochemical viability demonstration of cells seeded on Novocart 3D, the approximately 2-mm thick samples were incubated with 2 pM calcein and 0.2 pM ethidium homodimer at 37° C for 120 minutes (Live/Dead® assay kit; Molecular Probes, Eugene, OR, USA). Controls were treated with three freeze-thaw cycles in liquid nitrogen before the Live/Dead incubation.

The postoperative procedure depended on the location of treated defects. For the medial and lateral condyle and the tibial plateau, the patients were advised to partially weight-bear with 20 kg for 6 weeks. Afterward weightbearing could be increased 20 kg every second week until full weightbearing

was reached. ROM was not limited and continuous passive motion therapy started at the first postoperative day. Patients who had a MACT in the patellofemoral compartment were also instructed to partial weightbearing for 2 weeks and then full weightbearing was allowed. To avoid pressure on the implants, ROM was limited to 40° of flexion for 6 weeks and then increased 20° every second week. Restriction of ROM was controlled by patella-stabilizing braces. Patients received physiotherapy from the first postoperative day starting with passive movement for 6 weeks and then continuing with active training for another 6 weeks under therapy supervision. In the first postoperative year cycling and swimming were allowed; after 1 year, other sport activities were unrestricted.

Patients were assessed with physical examination 6 weeks, 3 months, and 1 year postoperatively. MRI was performed 3 months and 1 year postoperatively with special sequences for cartilage analysis according to a protocol by Trattnig et al. [24, 39]. Complication of all grades according to the classification of Dindo et al. [10] was distinguished during physical examination or with MRI at followup time points. Knee function was analyzed by the IKDC score at 3 months and 12 months postoperatively.

To analyze the change of IKDC measurements over time (preoperatively and 3 months and 12 months postoperatively), a repeated-measures analysis of variance was performed. The assumption of normality was proven by use of skewness (absolute value smaller than 1), kurtosis (absolute value smaller than 1), and visually by a histogram. In addition, Bonferroni-adjusted post hoc t-tests provided comparisons between the different time points. For the enzyme-linked immunosorbent assay test, a paired t-test was performed. All calculations were made with the software package SPSS Statistics 20.0 (IBM, Armonk, NY, USA).

Results

All MACT implants allowed deposition of extracellular matrix deposition under in vitro conditions. Histologically all 50 implants showed homogenous dispersion of chondrocytes in the porous part of the matrices and chondrogenic differentiation with extracellular matrix production during culture. The intensity of the glycosaminoglycan (Fig. 2) and collagen type II (Fig. 3) staining improved from Day 0 to Day 21. The cells in the implants produced extracellular matrix (Fig. 3) with a chondrogenic phenotype in all cases. Live/Dead® staining at Day 0 showed nearly exclusively vital cells (Fig. 4). In 25 cases analyzed by an enzyme-linked immunosorbent assay test for collagen II the collagen II, content subjectively appeared improved from Day 1 to Day 21. No sample failed to produce collagen II.

Fig. 2A–D Representative images of chondrogenic differentiation of implants for MACT of four different patients (**A–D**) shown in DMMB staining at different time points (Days 1, 7, 14, 21) starting at the day of transplantation. Implants of all 50 patients showed chondrogenic

differentiation with an increasing metachromatic staining in the collagen layer over time. The superficial layer represents the dense stabilizing pericardium layer (Stain; DMMB, original magnification, $\times 10$).

We observed an improvement ($p < 0.001$) of the median IKDC scores from preoperative to followup at 1 year: 41 points versus 67 points (Fig. 5). Seventeen of the 74 patients showed an improvement of less than 10 points. Eight of these 17 patients had a retropatellar defect. Of all 75 patients, six had transplant failure and three developed arthrofibrosis with a limited ROM. We observed no improvement in median knee function at 3 months

(IKDC = 44 points) with nearly the same IKDC level as preoperatively (Fig. 5).

Discussion

In recent years MACT has been reported in multiple studies for treating large cartilage defects [5, 27, 32, 34, 40].

Fig. 3A–D Representative immunohistochemical analysis of implants for MACT of four different patients (A–D) over time starting at the day of implantation. In collagen II staining, increasing

chondrogenic differentiation was seen in all cases from Day 1 to Day 21. The superficial layer represents the dense stabilizing pericardium layer (Stain, Collagen II, original magnification, $\times 10$).

Cell quality, chondrogenic differentiation of the implant, and quality of the repair tissue are crucial for restoration of knee function after MACT. Complete filling with well-

differentiated tissue correlates with improved knee function [34]. On the other hand, incomplete defect filling with undifferentiated scar tissue is associated with a risk of

Fig. 4A–B Cell viability evaluation in Live-Dead staining. On the left side (A) all green fluorescing cells in the implant are viable. On the right side (B) a three times frozen sample served as a control: red

fluorescing cells indicate dead cells (Stain, Live-Dead-Staining, original magnification, $\times 25$).

Fig. 5 IKDC scoring results preoperatively and 3 months and 12 months postoperatively after MACT of full-thickness cartilage defects. Improvement of the IKDC score can be seen from preoperative status to the 12-month postoperative results. The average improvement was 25.3 points. No difference was seen comparing the preoperative and the 3-month postoperative results.

persistent pain and lower function, particularly in larger chondral defects [14, 17, 21, 26]. Although the importance of a high quality of repair tissue is well described [27, 32, 34], little is known about the chondrogenic potential of implants for MACT at the time of implantation. We therefore determined whether (1) MACT implants allowed deposition of extracellular cartilage matrix in an in vitro culture model and (2) these implants were associated with

improved knee function 1 year after MACT in large cartilage defects.

We recognized limitations to our study. First, owing to the retrospective design, we had no control group. Consequently our findings cannot be directly compared with other treatment options or spontaneous regeneration. In a followup study of 84 patients with untreated focal full-thickness defects, Løken et al. [22] showed activity levels generally decreased over a median of 5 years. In a randomized trial, Basad et al. [5] found MACT was associated with superior functional restoration compared with MF. However, our intent was not to compare MACT with other forms of treatment, but rather to report the function of patients treated with MACT and to describe the repair potential of their implants. Second, we had a followup of only 1 year. Cartilage repair and tissue maturation may take longer. Using quantitative MRI, Trattinig et al. [38] suggested repair tissue global T2 values approached that of control sites after more than 1.5 years. However, the intention of the clinical part of the current study was to evaluate improvement of function in the short term after MACT. Third, our patient population was not homogenous because of the different locations, sizes, and etiologies of the defects. Some MACTs were combined with other procedures like ACL repair or osteotomy. The influence of the different procedures cannot be distinguished, but, as known from the literature, addressing all comorbidities is crucial for successful regenerative therapy.

We found a cartilage matrix deposition in all implants used for MACT. To analyze the implants, we further

cultivated cell-matrix constructs in a controlled in vitro culture model. After 21 days in chondrogenic medium, all samples showed chondrogenic differentiation with a histological increase in extracellular matrix deposition, positive immunohistochemical staining for collagen II, and an increase of collagen II content in the enzyme-linked immunosorbent assay test from Day 0 to Day 21. Pietschmann et al. [33] evaluated the transplant at the status of implantation and analyzed the cells regarding vitality and morphology. In their study, the total number of cells found on the implants did not correlate with the results of the IKDC score 1 year postoperatively. However, they found a negative correlation between the number of morphologic abnormal cells in the implant and the IKDC score. This suggests the quantity of morphologically normal cells in the implant is an important parameter for subsequent function [33]. In our study, the Live/Dead[®] staining showed mainly living cells. All implants revealed chondrogenic differentiation in vitro indicating the positive potential to regenerate cartilage defects. According to a number of authors [14, 17, 21, 26], macroscopic and histological insufficient repair tissue results in worse outcome and failure of ACT. In their systematic review, Vavken and Samartzis noted that defect filling, congruency of the surface, and integration of the repair tissue into the native surrounding cartilage were important subsequent functions [42]. Henderson et al. [14] and LaPrade et al. [21] showed that in revision surgery, symptomatic knees after ACT revealed macroscopic abnormal repair tissue with predominantly fibrocartilage-like tissue. Knowing the importance of appropriate quality of the chondrocytes in the transplant, Saris et al. [35] are using characterized chondrocytes and saw a correlation between cell quality and clinical outcome [35].

One year postoperatively, we found knee function in the IKDC score was improved over 20 points compared with the preoperative status. This is consistent with data from the literature, in which the IKDC score shows a similar increase after MACT [12, 19]. We found no improvement 3 months in the IKDC but the IKDC score is a functional knee score, so no improvement can be expected as a result of the long rehabilitation protocol with partial weight-bearing for up to 3 months. The average result 1 year postoperatively was 67, which meant an improvement of 26 points. However, these data also show that the IKDC levels after MACT are still below the reference values of healthy subjects [13]. Nevertheless, Harris et al. [12] suggested full-thickness cartilage defects more than 4 cm² are best and most reliable treated by MACT. In a randomized controlled study, Basad et al. [5] analyzed the outcome of large chondral defects comparing ACT with MF. Particularly in large defects, ACT resulted in improved knee function compared with MF. Kon et al. [18] reported active and athletic patients appear to benefit more from therapy

with autologous chondrocytes in comparison to MF with higher Knee Injury and Osteoarthritis Outcome Score and IKDC scores after ACT compared with MF. There are nonetheless some circumstances in which treatment of large cartilage defects remains problematic: although patients with repaired defects on the medial condyle or the trochlea may experience an increase in knee function, treatment of retropatellar defects reportedly has a more variable and overall reduced outcome [1, 7, 27–29]. The particular anatomical and biomechanical situation may explain the lower scores. Five of seven of the patients of Brittberg et al. [8] undergoing retropatellar ACT defect treatment showed only poor or fair results in clinical evaluation. However, in our study, more than 60% showed improvement of more than 20 points in IKDC scoring, which is consistent with two studies of second- and third-generation ACT [33, 34] showing substantial improvement in function. Therefore, retropatellar cartilage defects can be treated with MACT; however, patients should be informed about the higher risk of failure. Additionally, identification and correction of comorbidities of the patella and the extensor mechanism of the knee are crucial and necessary to achieve appropriate outcome. Vasiliadis et al. [41] recently reported reasonable improvement in Lysholm scores of patients with patellofemoral lesions when patellar malalignment or instability was corrected.

In conclusion, implants for MACT showed adequate in vitro chondrogenic potential and implant quality with extracellular cartilage deposition in all cases. Clinically the majority of patients treated with these implants had improved function 1 year postoperatively in the IKDC score.

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