

# Osteoarthritis and Cartilage



## T2-relaxation time of cartilage repair tissue is associated with bone remodeling after spongiosa-augmented matrix-associated autologous chondrocyte implantation



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### SUMMARY

**Objective:** To investigate whether T2 relaxation time measurements of cartilage repair tissue and structural changes of the knee joint are associated with subchondral bone architecture after spongiosa-augmented matrix-associated autologous chondrocyte implantation (MACI).

**Design:** Both knees of 25 patients ( $25.5 \pm 7.8$  y; 10 women) were examined preoperatively and 2.7 years after unilateral spongiosa-augmented MACI with 3T magnetic resonance (MR) imaging. Cartilage composition was assessed using T2 relaxation time measurements, subchondral trabecular bone microstructure was quantified using a 3D phase-cycled balanced steady state free-precession sequence. Structural knee joint changes were assessed using the modified Whole-Organ Magnetic Resonance Imaging Score (WORMS). The Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) score was used for the postoperative description of the area that underwent MACI. Correlations were assessed using Spearman's rank correlation coefficients.

**Results:** Hypertrophy of the cartilage repair tissue was found in 2 of 25 patients, both after a MACI procedure at the patella, 21 patients showed congruent filling. In subchondral bone of the cartilage repair compartment, apparent trabecular thickness was significantly higher in compartments with elevated cartilage T2 ( $n = 17$ ;  $0.37 \pm 0.05$  mm) compared to those showing no difference in cartilage T2 compared to the same compartment in the contralateral knee ( $n = 8$ ;  $0.27 \pm 0.05$  mm;  $P = 0.042$ ). Significant correlations were found between the overall progression of WORMS and the ipsilateral vs contralateral ratio of average trabecular thickness ( $r = 0.48$ ,  $P = 0.031$ ) and bone fraction ( $r = 0.57$ ,  $P = 0.007$ ).

**Conclusions:** After spongiosa-augmented MACI, T2 values of cartilage repair tissue and structural knee joint changes correlated with the quality of the underlying trabecular bone.

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### Introduction

Cartilage defects caused by traumatic injuries may accelerate the development of osteoarthritis in young patients<sup>1</sup>. Once cartilage damage has occurred, articular cartilage is unable to regenerate the same hyaline matrix<sup>2</sup>. Therefore, a number of cartilage repair techniques, including osteochondral transplantation and matrix-associated autologous chondrocyte implantation (MACI), have been developed<sup>2–5</sup>, in order to repair articular cartilage in cases of focal cartilage defects and to slow the onset of osteoarthritis<sup>6,7</sup>.

Good to excellent clinical outcome as well as radiological outcome, assessed using morphological magnetic resonance (MR) sequences and radiographs, have been reported after cartilage repair with the MACI procedure<sup>6,8–11</sup>; few studies have evaluated outcome of patients that had undergone MACI with quantitative MR imaging<sup>12–14</sup>. It is known, that cartilage collagen content decrease and water content increase cause prolonged T2 relaxation times and therefore elevated cartilage T2 values on T2 maps<sup>15</sup>. Therefore, T2 relaxation time measurements, as one of the quantitative MR imaging techniques, is known to reflect cartilage quality.

More recently, MACI was also introduced in combination with spongiosa augmentation of the subchondral bone for treatment of osteochondral defects, which previously required osteochondral transplantation in order to repair bony defect, however, so far only one study assessed this combination of spongiosa graft and autologous matrix-induced chondrogenesis in the talus<sup>16</sup>. Moreover, the quantitative assessment of cartilage and subchondral bone architecture after cartilage repair has not been assessed after MACI until today.

Therefore, purpose of this study was to investigate the osteochondral maturation after spongiosa-augmented MACI in the knee and whether the quality of the cartilage repair tissue is associated with the architecture of the underlying subchondral bone, as assessed with quantitative MR imaging.

## Methods

### Subjects

Between September 2008 and August 2013, unilateral, uni-compartmental MACI was performed at the patella or at the medial or lateral femoral condyle of the knee joint in 42 consecutive patients. The patient selection is shown in Fig. 1. Exclusion criteria for this study were previous surgery at the ipsilateral knee ( $n = 1$ ) and surgery at the contralateral knee ( $n = 6$ ). Ten patients were not willing to participate in the study. The remaining patients ( $n = 25$ ; 10 women, mean age  $25.5 \pm 7.8$  years) did not present MR imaging contraindications and were included in the study. None of the included patients had subsequent surgery at the ipsilateral knee. Clinical assessment and MRI of the treated knee was performed preoperatively and after 2.7 years. The patient characteristics of the patients excluded from this study ( $N = 17$ ) did not differ significantly compared to the patient characteristics of the patients included in this study (7 women, mean age  $28 \pm 9.2$  years,  $P > 0.05$  for each comparison). None of the patients included in this study underwent treatment with medication which may have affected bone or cartilage biology at the time of inclusion in the study and at 2.7-year

follow-up. The study was approved by the local institutional review board (Ethikkommission der Fakultät für Medizin der Technischen Universität München, Munich, Germany). All patients gave written and informed consent prior to the participation in the study.

An a priori power analysis was performed to calculate the appropriate size of study cohorts in order to analyze differences between the knee that underwent MACI and the contralateral knee. Using data of our previous study, the mean difference ( $\pm$ standard deviation) between the ipsilateral knee and the contralateral knee of cartilage T2 averaged over all compartments was  $1.7 \pm 2.9$  ms. With these data a comparison of the ipsilateral and contralateral was simulated and we determined a sample size of at least 25 patients would achieve a power  $>0.8$ <sup>17</sup>. Therefore, the size of the assess cohort ( $N = 25$  patients) was sufficient for the analysis.

### Surgery

All included patients received spongiosa-augmented MACI either at the patella or at the medial or lateral femoral condyle. In brief, the treatment consisted of an initial arthroscopic surgery (as the first intervention of this two-step procedure) during which healthy articular cartilage was harvested via biopsy from a non-weight-bearing region, either from the trochlear notch or from the medial or lateral femur condylar ridge<sup>18</sup>. Following, chondrocytes were isolated from the cartilage tissue. Chondrocytes were passaged in a medium, cultured for approximately 6–8 weeks and seeded onto a biphasic membrane composed of collagen and chondroitin sulfate before implantation. At the second stage of the procedure, the implantation, open arthrotomy was performed. Spongiosa plugs were harvested from either the distal femur or from the iliac crest and implanted into the bony defect. Then, the MACI membrane was shaped according to the cartilage defect. The membrane surface was dried and sealed using a final fibrin glue application to make in order to improve stability and in order to create a water proof surface.

Postoperatively, reduced weight bearing to 20 kg was required for 6 weeks with following weekly increases of weight bearing. All patients underwent physiotherapy for at least 3 months.

### MR imaging

Morphological and quantitative MR imaging of both knees was performed 2.7 years after unilateral surgery at a 3T MR scanner (Ingenia, Philips Healthcare, Best, the Netherlands) using a dedicated 8-channel knee coil (Medical Advances Milwaukee WI, USA). MR sequence parameters are displayed in Table 1. For the quantitative assessment of cartilage composition, a sagittal 2D multislice multi echo (MSME) spin echo T2-weighted (w) sequence was acquired. The employed T2 mapping sequence was a modification of the T2 mapping sequence from the imaging protocol used in the multi-center Osteoarthritis Initiative (OAI) study<sup>19</sup>. The sequence acquired 6 echoes at echo times (TE) = 10, 20, 30, 40, 50 and 60 ms. However, the first echo of the multi-echo spin echo sequence at TE = 10 ms was excluded from the T2 fitting. The maximum TE was 60 ms. The longest TE image had therefore adequate signal-to-noise ratio (SNR).

A three-dimensional phase-cycled balanced steady-state free precision (bSSFP) sequence was used in order to quantify the subchondral bone structure.

### Morphological MR assessment

Morphologic MR images acquired preoperatively and 2.7 years after surgery were evaluated independently by two radiologists

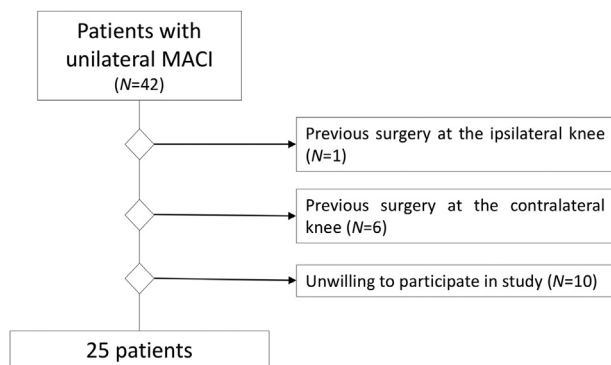


Fig. 1. Flow chart illustrating patient selection.

**Table 1**  
MR sequence parameters

| Sequence                               | IM-w TSE  | IM-w TSE  | T1-w TSE  | bSSFP     | MSME SE T2             |
|----------------------------------------|-----------|-----------|-----------|-----------|------------------------|
| Additional features                    | 2D        | 2D        | 2D        | 2D        | 2D                     |
| Plane                                  | Cor       | Sag       | Sag       | Cor       | Sag                    |
| Echo time/step (TE; ms)                | 44        | 44        | 13        | 3         | 10, 20, 30, 40, 50, 60 |
| Repetition time (TR; ms)               | 3363      | 4202      | 785       | 8         | 2200                   |
| Field of view (FOV; mm)                | 140       | 140       | 140       | 100       | 140                    |
| Slice thickness (mm)                   | 3         | 3         | 3         | 1         | 2.5                    |
| In-plane resolution (mm <sup>2</sup> ) | 0.4 × 0.4 | 0.4 × 0.4 | 0.4 × 0.4 | 0.2 × 0.2 | 0.4 × 0.4              |
| Flip angle (°)                         | 90        | 90        | 90        | 40        | 90                     |
| Number of slices                       | 24        | 30        | 28        | 200       | 30 (per echo)          |
| Slice distance (mm)                    | 3.6       | 3.6       | 3.6       | 0.5       | 2.5                    |
| Echo Train Length                      | 9         | 9         | 3         | 1         | 5                      |
| Bandwidth per pixel (Hz)               | 187       | 187       | 143       | 244       | 251                    |
| Phase Encoding Direction               | F/H       | F/H       | F/H       | R/L       | F/H                    |
| SENSE reduction factor                 | 1.7       | 1.7       | 1.5       | 2         | 2                      |
| Number of averages                     | 2         | 2         | 1         | 2         | 1                      |
| Acquisition time (min)                 | 4:50      | 4:50      | 3:06      | 6:42      | 5:33                   |
| Fat saturation                         | yes       | yes       | no        | no        | no                     |

(ASG and BJS, 6 years of experience) using the semi-quantitative scoring systems as described below. Radiologists were blinded for clinical information and imaging findings at other time points. In cases of disagreement a consensus reading was performed.

#### MOCART score

The MR observation of cartilage repair tissue (MOCART) score was used for the postoperative description of the area that underwent MACI<sup>20</sup>, assessing the degree of filling of the defect, the integration to the adjacent border zone, the surface of the repair tissue and bone surface as well as the subchondral bone.

#### Whole-Organ Magnetic Resonance Imaging (WORMS) score

Pre- and postoperative morphological MR sequences of the ipsilateral knees were assessed using the semi-quantitative modified WORMS grading system, as previously described<sup>21,22</sup>. For each subscale a sum score was calculated by adding the scores of all subregions. A total WORMS score was estimated by summing up all sum scores.

In addition, the size of the defect that was consequently treated with spongiosa-augmented MACI was measured on preoperative images.

#### Quantitative analyses

For trabecular bone analysis and cartilage analysis, segmentations were performed by two readers (GF and ASG), both under the supervision of an experienced radiologist (PJM, 10 years of experience).

#### Histomorphometric trabecular bone analyses

For the trabecular bone analysis, an in-house interface description language (IDL)-based program was used, as described previously<sup>23</sup>. OsiriX Lite 7.0.2 (Pixmeo, Bernex, Switzerland) was used for manual segmentation of the subchondral bone in the patella (PAT), trochlea (TRO), medial femoral condyle (MF), lateral femoral condyle (LF), medial tibia (MT), lateral tibia (LT), based on coronal bSSFP sequences. Regions of interest (ROI) were drawn on the coronal images, on 20 consecutive sections from anterior to posterior<sup>23–26</sup>. The height of the ROI of the segmented subchondral bone was 1 cm. For the segmentation of the subchondral bone of the patellofemoral joint, axial reconstructions were obtained from

the bSSFP datasets and segmented on 20 consecutive sections from the proximal to the distal subchondral region (Fig. 2). For the calculation of the trabecular bone parameters, a dual threshold algorithm was used<sup>24–26</sup>. Histomorphometric parameters were then calculated using a mean intercept length method and the following values were calculated from the sequence<sup>23</sup>: apparent bone fraction (BF), apparent trabecular number (Tb.N), apparent trabecular separation (Tb.Sp), and apparent trabecular thickness (Tb.Th). This analysis was performed for the ipsilateral and contralateral knee.

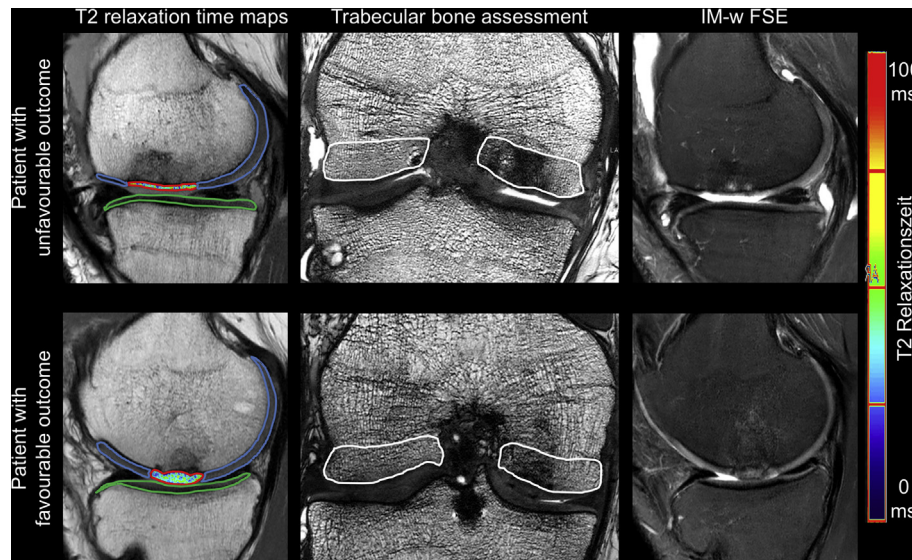
#### T2 relaxation time measurements

For the analyses of the cartilage T2 relaxation times, T2 relaxation time maps were calculated from 2D T2 MSME standard error (SE) images using a mono-exponential nonnegative least square fit analysis (27). Five compartments were analyzed at the ipsilateral and the contralateral knee (PAT, MF, LF, MT, LT). The region of the cartilage repair tissue at the PAT, MF or LF was segmented separately from the surrounding tissue of the specific knee compartment (Fig. 2). The ROI of the cartilage repair region was mirrored on the contralateral knee and was excluded from the compartmental measurements of the contralateral knee.

Moreover, mean values of all analyzed compartments, excluding the MACI site, were calculated for both knees. A T2 ratio between the cartilage T2 of the ipsilateral and contralateral knee was assessed for the analysis in order to take the large inter-individual variability of T2 values into account, as previously described<sup>27,28</sup>. Increased T2 relaxation times in repair tissue compared to the cartilage of the contralateral knee implies a stronger anisotropy of the collagen matrix and increased water content in respective cartilage areas<sup>15</sup>. Therefore, high T2 values of the cartilage repair region may indicate an impairment of cartilage quality in the repaired region or may suggest a difference regarding the integration of the cartilage transplant in the surrounding tissue. Consequently, an analysis of cartilage regions with high and low T2 ratios was assessed separately. The cut off was an increase by two standard deviations of the matching contralateral cartilage region.

#### Clinical evaluation

For the evaluation of the clinical outcome 2.7 years after surgery, the knee injury and Osteoarthritis Outcome score (KOOS)<sup>29</sup> was used assessing knee pain, symptoms other than pain, activities of daily living (ADL), sport and recreation and related quality of life.



**Fig. 2.** Exemplary MR images and T2-maps of a 24-year-old male patient with high subchondral trabecular thickness at the osteochondral repair site (upper row) compared to the contralateral knee and a 22-year old male patient with low trabecular thickness at the osteochondral repair site (lower row) compared to the contralateral knee 2.7 years after MACI. The ROI segmenting the cartilage of the cartilage repair region (red) the medial femur condyle (blue) and the medial tibia (green) were mirrored to the contralateral knee for the T2 analysis. Trabecular bone structure post-processing: bone and marrow ROI were outlined for the lateral and medial femur condyle (white).

### Statistical analysis

Statistical processing was performed using SPSS 23 (IBM, Armonk, NY, USA) (PMJ, TB). All tests were performed based on a two-sided 0.05 level of significance. Differences in characteristics between patients that participated in the study and patients that did not participate in this study were evaluated by using one-way analysis of variance (parametric testing) and Chi-squared test (categorical variables). For the assessment of the linear relationships between the body mass index (BMI) and total MOCART score as well as the BMI and KOOS score, linear regression analyses were performed, with  $b$  as standardized coefficient.  $b$  indicates the amount by which the mean/average of the dependent variable changes if the independent variable is changed by one standard deviation keeping other independent variables constant.

The values of cartilage T2 are presented as mean  $\pm$  SE. Normal distribution of T2 relaxation times was confirmed using Kolmogorov–Smirnov and Shapiro–Wilk tests. Cartilage T2 values were compared between the knee that underwent MACI (ipsilateral) and the contralateral knee in the same patient using paired  $t$ -tests. All included patients had datasets for both knees and for the statistical analyses performed in this study, the unit of the analyses was the patient. Non-parametric data are presented as median and interquartile range. Correlations between semi-quantitative imaging subscores (MOCART at follow-up, WOMBS at follow-up, WOMBS progression (=WOMBS at follow-up – WOMBS at baseline) and trabecular parameters were assessed by using Spearman's rank correlation coefficient. The differences regarding the frequency of worsening between patellofemoral and tibiofemoral joint were assessed using Chi-squared tests with worsening defined as a change in WOMBS  $>0$ . Cartilage T2 values in the compartments undergoing MACI (PAT, MF, LF) that were higher than those of the corresponding compartment on the contralateral knee were considered as elevated. The Kolmogorov–Smirnov test indicated for the subchondral trabecular bone parameters no normal distribution ( $P < 0.05$ ). Therefore, differences of the subchondral trabecular bone parameters between the group with elevated and normal cartilage T2 values were assessed using the Mann–Whitney  $U$  test.

### Reproducibility

For intrareader reproducibility analysis, the same reader performed repeated WOMBS and MOCART gradings in 4 randomly selected patients for each time point with readings separated by at least 14 days. Inter-reader reproducibility was assessed based on all subjects separately for each time point. Intra-class correlation coefficients (ICCs) were calculated for MOCART as well as each WOMBS subscore. Intra- and interreader reproducibility were calculated for trabecular measurements and for cartilage T2 values on a percentage basis as the root mean square coefficient of variation (CV) to assess the reproducibility error, as previously described<sup>30</sup>. For the reproducibility analyses, segmentations each segmentation in each patient was performed once by each reader (ASG and GF) and for intra-reader reproducibility the readings were repeated by each reader in 10 patients. Results are shown in the [Supplemental data](#).

### Results

#### Patient and lesion characteristics

MACI was performed at the medial femur condyle ( $n = 14$ ), at the patella ( $n = 8$ ) and at the lateral femur condyle ( $n = 3$ ). The median size and range of the cartilage defects measured on MRI was  $4.1 \text{ cm}^2$  (range  $1.5\text{--}7.0 \text{ cm}^2$ ). All patients ( $n = 25$ ) presented an osteochondral lesion and the average depth of the bone defect was  $3.47 \pm 1.98 \text{ mm}$  and the average length of the bone lesion was  $13.42 \pm 6.68 \text{ mm}$ . The intraoperatively measured cartilage lesion size was  $3.8 \text{ cm}^2$  (range  $1.8\text{--}8.8 \text{ cm}^2$ ). The average BMI was  $25.8 \pm 4.5 \text{ kg/m}^2$ . The follow-up time averaged over all patients was  $2.7 \pm 0.3 \text{ years}$  (range: from 2.4 to 3.0 years).

#### Cartilage T2 relaxation times and subchondral trabecular analyses

When comparing the cartilage T2 values of the ipsilateral with the contralateral knee 2.7 years after the patella MACI procedure ( $n = 8$ ), cartilage T2 values at the trochlea were significantly higher in the ipsilateral compared to the contralateral knee (mean



difference  $2.81 \pm 0.85$ ,  $P = 0.03$ ; Table II). Moreover, after MACI at the femoral condyles ( $n = 17$ ), there were no significantly elevated T2 values found in the compartments of the ipsilateral knee compared to the corresponding compartments of the contralateral knee (Table III). The T2 values and trabecular analysis values are shown as Supplemental data.

A significant correlation was found between the ratio of global T2 of the ipsilateral and contralateral knee and the ratio between the Tb.Th in the spongiosa-augmented region and the contralateral region ( $r = 0.47$ ,  $P = 0.032$ ). An analogous significant correlation was between the ratio of T2 of MACI regions and the corresponding contralateral regions and the ratio between the Tb.Th in the spongiosa-augmented regions and the contralateral regions ( $r = 0.36$ ,  $P = 0.031$ , Table IV). The ratio of the ipsilateral and the contralateral Tb.N in the cartilage repair region correlated significantly with the ratio between global T2 values of the ipsilateral and contralateral knee ( $r = 0.44$ ,  $P = 0.041$ ). Moreover, the ratio of the BF ( $P = 0.026$ ) and Tb.N ( $P = 0.015$ ) of the ipsilateral and contralateral knee were significantly elevated in spongiosa-augmented areas with high T2 values in the ipsilateral overlying MACI repair

tissue (versus T2 values of the corresponding area in the contralateral knee) compared to patients with equal or lower T2 values (Fig. 3).

#### Trabecular parameters and morphological knee joint assessment

A significant correlation was found between the change over time in total WORMS and the ratio between the global ipsilateral and contralateral knee of the Tb.Th ( $r = 0.48$ ,  $P = 0.031$ ; Table V), BF ( $r = 0.57$ ,  $P = 0.007$ ) and Tb.N ( $r = 0.48$ ,  $P = 0.034$ ). The same effect was found for change in the subscore WORMS meniscus (ratio Tb.Th,  $P = 0.001$ ) and cartilage (ratio BF,  $P = 0.004$ ).

The integration of cartilage repair tissue in the surrounding cartilage as depicted using the MOCART score correlated negatively significantly with the ratio of the Tb.Th and with the ratio of the BF ( $r = -0.46$ ,  $P = 0.031$  and  $r = -0.49$ ,  $P = 0.022$ ; Table V and Supplemental data). No significant correlations were found between the clinical outcome as assessed with KOOS and the histomorphometric trabecular bone parameters ( $P > 0.05$  for all).

**Table II**

Cartilage T2 relaxation time values of the ipsilateral and contralateral knee after unilateral spongiosa-augmented MACI at the **patella** ( $n = 8$ ). T2 values are presented for each individual joint compartment and for the global knee joint. MF = medial femoral condyle; LF = lateral femoral condyle; MT = medial tibia; LT = lateral tibia; PAT = patella (without the cartilage repair region); TRO = trochlea; CR = cartilage repair region

| T2 relaxation time (ms) | Ipsilateral compartment | Std. Error Mean | Contralateral compartment t | Std. Error Mean | P-value*    |
|-------------------------|-------------------------|-----------------|-----------------------------|-----------------|-------------|
| MT                      | 30.12                   | 1.77            | 33.48                       | 1.40            | 0.13        |
| LT                      | 30.01                   | 1.67            | 32.69                       | 1.18            | 0.06        |
| MF                      | 37.55                   | 0.78            | 38.23                       | 1.09            | 0.65        |
| LF                      | 39.79                   | 1.10            | 39.78                       | 0.98            | 1.00        |
| CR                      | 35.72                   | 1.76            | 33.31                       | 2.24            | 0.39        |
| TRO                     | 42.14                   | 1.73            | 39.33                       | 1.22            | <b>0.03</b> |
| PAT                     | 33.88                   | 0.92            | 33.41                       | 1.73            | 0.34        |
| Global                  | 36.09                   | 0.85            | 36.66                       | 0.92            | 0.23        |

\*P-values <0.05 are in bold.

**Table III**

Cartilage T2 relaxation time values of the ipsilateral and contralateral knee after unilateral spongiosa-augmented MACI at the **medial or lateral femur condyle** ( $n = 17$ ). T2 values are presented for each individual joint compartments and for the global knee joint. MF = medial femoral condyle (without the cartilage repair region, if applicable); LF = lateral femoral condyle (without the cartilage repair region, if applicable); MT = medial tibia; LT = lateral tibia; PAT = patella; TRO = trochlea; CR = cartilage repair region

| T2 relaxation time (ms) | Ipsilateral compartment | Std. Error Mean | Contralateral compartment | Std. Error Mean | P-value*    |
|-------------------------|-------------------------|-----------------|---------------------------|-----------------|-------------|
| MT                      | 33.10                   | 0.87            | 34.43                     | 2.63            | 0.16        |
| LT                      | 31.16                   | 1.34            | 35.11                     | 1.09            | <b>0.01</b> |
| MF                      | 41.74                   | 0.88            | 41.49                     | 1.17            | 0.90        |
| LF                      | 41.06                   | 0.99            | 39.65                     | 1.15            | 0.18        |
| CR                      | 42.21                   | 1.28            | 43.45                     | 1.16            | 0.60        |
| TRO                     | 40.13                   | 1.03            | 40.80                     | 0.98            | 0.60        |
| PAT                     | 34.37                   | 0.80            | 36.01                     | 0.92            | 0.10        |
| Global                  | 37.04                   | 0.67            | 38.53                     | 0.68            | 0.06        |

\*P-values <0.05 are in bold.

**Table IV**

Correlations of the cartilage T2 ratio of the ipsilateral and the corresponding contralateral knee compartments with the respective ratio of the trabecular bone parameter ratios of each compartment. MF = medial femoral condyle; LF = lateral femoral condyle; MT = medial tibia; LT = lateral tibia; PAT = patella; TRO = trochlea; CR = cartilage repair region

|                              |                         | Ratio between ipsilateral and contralateral cartilage T2 value of each knee compartment |       |       |       |       |              |       |       |
|------------------------------|-------------------------|-----------------------------------------------------------------------------------------|-------|-------|-------|-------|--------------|-------|-------|
|                              |                         | Global T2                                                                               | MT    | LT    | MF    | LF    | CR           | TRO   | PAT   |
| Trabecular Thickness (Tb.Th) | Correlation coefficient | <b>0.47</b>                                                                             | 0.21  | -0.07 | 0.42  | 0.05  | <b>0.36</b>  | 0.20  | 0.31  |
|                              | P-value*                | <b>0.032</b>                                                                            | 0.38  | 0.76  | 0.06  | 0.82  | <b>0.031</b> | 0.39  | 0.18  |
| Trabecular Space (Tb.Sp)     | Correlation coefficient | 0.27                                                                                    | 0.03  | 0.04  | 0.03  | 0.51  | 0.20         | 0.15  | 0.24  |
|                              | P-value*                | 0.24                                                                                    | 0.92  | 0.85  | 0.92  | 0.02  | 0.39         | 0.52  | 0.31  |
| Bone fraction                | Correlation coefficient | -0.29                                                                                   | -0.04 | 0.09  | -0.14 | -0.06 | -0.35        | -0.20 | -0.27 |
|                              | P-value*                | 0.21                                                                                    | 0.86  | 0.71  | 0.55  | 0.80  | 0.12         | 0.39  | 0.26  |
| Trabecular number (Tb.N)     | Correlation coefficient | -0.21                                                                                   | -0.05 | 0.12  | -0.11 | -0.05 | <b>0.44</b>  | -0.22 | -0.25 |
|                              | P-value*                | 0.36                                                                                    | 0.85  | 0.60  | 0.63  | 0.84  | <b>0.041</b> | 0.33  | 0.29  |

\*P-values <0.05 are in bold.

### Morphological outcome

The average MOCART score was  $67.7 \pm 18.2$  points. Full thickness defects within the MACI region or complete delamination of the entire transplant were found in 2 of 25 patients (one with a MACI procedure at the medial femur condyle and one with a MACI procedure at the patella). Hypertrophy of the cartilage repair tissue was found in 2 of 25 patients, both after a MACI procedure at the patella. The remaining patients ( $n = 21$ ) showed congruent filling of the MACI region.

A statistical trend was found for the association between preoperative BMI and total MOCART score ( $b = -0.4$  (95% confidence interval  $-0.5, 0.1$ ;  $P = 0.09$ ; [Supplemental data](#)). Nevertheless, there was no significance found when assessing the association between the preoperative BMI and the clinical symptoms assessed with the KOOS score ( $P = 0.62$ ). A higher preoperative BMI was associated with presence of knee joint effusion in the mid-term postoperative follow-up ( $b = -0.4$ , (95% confidence interval  $-0.5, 0.0$ ;  $P = 0.046$ ).

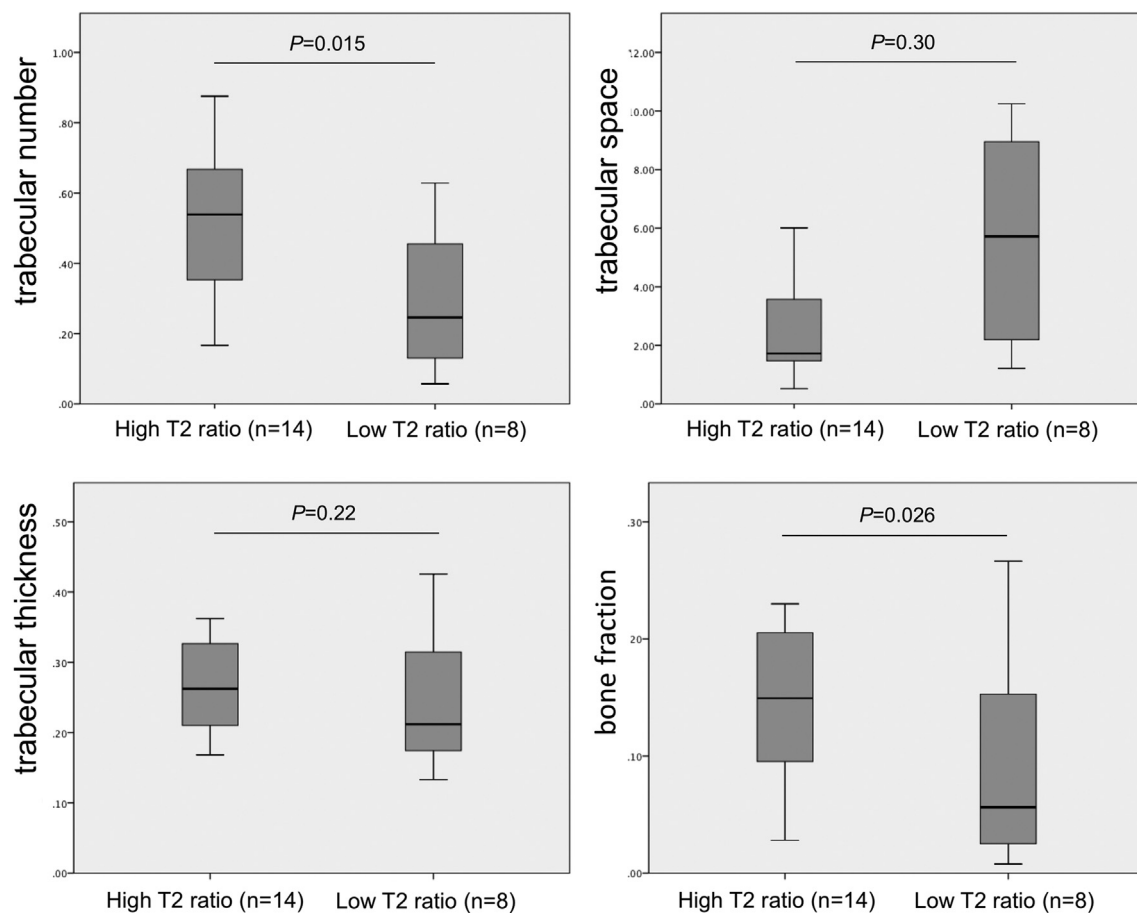
Over 2.7 years, total WORMS only showed a subtle progression (median total WORMS 3.0, interquartile range 2.00–6.00; [Supplemental data](#)). Cartilage WORMS showed overall low rates of cartilage degeneration within 2.7 years after MACI (WORMS increase cartilage between baseline and 2.7 year follow up: median WORMS cartilage progression 2.50, interquartile range 2.00–4.00).

With respect to the localization of MACI, worsening of cartilage lesions, assessed with the subscore WORMS cartilage, was only substantial in the subgroup that underwent MACI procedure at the patella compared to patients that underwent MACI at the tibiofemoral joint (median 2.50, interquartile range 2.00; 3.50 vs median 1.5, interquartile range 0.5–2.50) and worsening of cartilage lesions was significantly more often found at the patellofemoral joint than at the tibiofemoral joint in these patients ( $P = 0.036$ ).

### Discussion

In this study, we have shown significant correlations between the cartilage quality of compartments treated with MACI, as evaluated with quantitative T2 relaxation time mapping, and structural parameters describing the regeneration and integration of subchondral trabecular bone. Moreover, we have shown that the overall cartilage quality as well as the longitudinal, postoperative development of degenerative joint changes are correlated with these subchondral trabecular bone parameters. Therefore, cartilage repair tissue quality may depend on the proper regeneration of the underlying trabecular bone.

In contrast to isolated cartilage defects, osteochondral defects require not only cartilage repair but also repair of the underlying bony defect. Therefore, osteochondral defects were mostly treated using Osteochondral autograft transfer system (OATS) techniques<sup>31</sup>.



**Fig. 3.** Comparison of the average ratio of trabecular parameters at the osteochondral repair site (ipsilateral/contralateral knee) between patients with a high cartilage T2 ratio (ipsilateral cartilage T2/contralateral cartilage T2,  $n = 14$ ) and patients with low cartilage T2 ratio ( $n = 11$ ) in the cartilage repair region 2.7 years after MACI. The threshold to distinguish patients with high and low T2 values in the repair tissue was defined as the mean T2 value of the matching contralateral cartilage region plus two standard deviations. Average ratio of trabecular parameters, upper and lower boxes indicating the 1. and 3. quartile of the ratios and upper and lower whisker indicating the range of the ratios of the trabecular parameters.

**Table V**

Correlation of the ratio of the trabecular bone parameters of the ipsilateral and contralateral knee within the MACI region with change in WORMS subscore from preoperatively to 2.7 years postoperatively after spongiosa-augmented MACI (total WORMS, meniscus WORMS, cartilage WORMS, bme WORMS) as well as with MOCART subscores (filling MOCART, integration MOCART, surface MOCART, total MOCART)

| Change in WORMS subscore over 2.7 years after MACI |                         | Trabecular Thickness (Tb.Th) | Trabecular Space (Tb.Sp) | Bone fraction (BF) | Trabecular number (Tb.N) |
|----------------------------------------------------|-------------------------|------------------------------|--------------------------|--------------------|--------------------------|
| Total                                              | Correlation coefficient | <b>0.48</b>                  | −0.22                    | <b>0.57</b>        | <b>0.48</b>              |
|                                                    | P-value*                | <b>0.031</b>                 | 0.34                     | <b>0.007</b>       | <b>0.034</b>             |
| Meniscus                                           | Correlation coefficient | <b>0.65</b>                  | −0.10                    | −0.04              | 0.03                     |
|                                                    | P-value*                | <b>0.001</b>                 | 0.66                     | 0.85               | 0.90                     |
| Cartilage                                          | Correlation coefficient | 0.42                         | −0.06                    | <b>0.60</b>        | 0.42                     |
|                                                    | P-value*                | 0.06                         | 0.81                     | <b>0.004</b>       | 0.06                     |
| BME                                                | Correlation coefficient | −0.36                        | −0.274                   | <b>0.49</b>        | <b>0.49</b>              |
|                                                    | P-value*                | 0.11                         | 0.23                     | <b>0.031</b>       | <b>0.022</b>             |
| MOCART Score                                       |                         |                              |                          |                    |                          |
| Filling MOCART                                     | Correlation coefficient | −0.09                        | 0.25                     | −0.27              | −0.34                    |
|                                                    | P-value*                | 0.69                         | 0.25                     | 0.2                | 0.11                     |
| Integration MOCART                                 | Correlation coefficient | <b>−0.46</b>                 | 0.38                     | <b>−0.49</b>       | −0.41                    |
|                                                    | P-value*                | <b>0.03</b>                  | 0.08                     | <b>0.02</b>        | 0.05                     |
| Surface MOCART                                     | Correlation coefficient | −0.39                        | 0.37                     | −0.39              | −0.30                    |
|                                                    | P-value*                | 0.06                         | 0.08                     | 0.07               | 0.16                     |
| Total MOCART                                       | Correlation coefficient | −0.30                        | 0.16                     | −0.23              | −0.11                    |
|                                                    | P-value*                | 0.16                         | 0.46                     | 0.28               | 0.61                     |

\*P-values <0.05 are in bold.

However, the indication for OATS plugs is limited by the defect size. Previous studies have shown good clinical outcomes after MACI also for large cartilage defects at the knee joint, however, integrity of the subchondral lamina is required for this technique<sup>18,32</sup>. MACI has now been combined with additional spongiosa augmentation in order to treat osteochondral lesions with large underlying bony defects.

Therefore, aim of this study was to qualitatively and quantitatively assess the progression of knee joint degeneration within 2.7 years after spongiosa-augmented MACI surgery with a focus on the quantitative assessment of the cartilage repair tissue and subchondral bone architecture. Our results showed higher rates of degeneration of the knee joint in patients with higher trabecular thickness and a higher BF in the osteochondral repair region. Especially cartilage and meniscal degeneration averaged over all compartments were significantly higher in patients with higher BF at the osteochondral repair site. Also, the integration of the MACI graft was significantly worse (according to MOCART score) and T2 values of the cartilage repair tissue were increased in patients with thicker trabeculae and a higher BF ratio at the osteochondral repair site. These results may suggest an interaction of the subchondral bone with the cartilage layer and are in line with a previous study showing interactions among bone, bone marrow and cartilage in the progression of knee joint degeneration<sup>33</sup>. Higher T2 values, indicating an insufficient cartilage matrix regeneration within the cartilage repair tissue, were found in patients with a higher BF and trabecular number 2.7 years after the MACI procedure. Consequently, a proper matrix formation, matrix integration and maturation may depend on regeneration and integration of subchondral trabecular bone. One pathophysiological explanation for this finding may be a reduced blood supply for cartilage in regions within bone regions with thicker trabeculae or even sclerotic bone<sup>34</sup>. Also, it needs to be mentioned that thicker trabeculae or sclerotic bone may also occur secondary, as reaction to increased stress on the subchondral bone due to the impaired quality of the cartilage repair tissue. This hypothesis is supported by previous studies showing that degenerative changes of the subchondral bone depend on the quality of the adjacent cartilage<sup>33,35</sup>. Correlations between cartilage T2 and subchondral bone changes were assessed in order to assess associations between cartilage maturation and the underlying subchondral bone. The results may indicate the necessity to reconstruct the subchondral bone in patients with a osteochondral defect, since the healing of the underlying subchondral bone may improve the outcome of cartilage maturation in patients that undergo the MACI procedure.

Moreover, after transplantation of spongiosa and cartilage repair, a further reason for a successful MACI procedure could be the structure and quality of the bone prior to surgery. In this study the quantitative subchondral bone parameters were only acquired postoperatively, which is a major limitation of this study. The morphological preoperative MR images revealed no pathologies in the remaining bone aside from the extent of the bony defect, therefore further studies with preoperative quantitative imaging are needed in order to assess the potential influence of these parameters on the outcome of the MACI procedure.

Moreover, the integration of the MACI in the surrounding cartilage showed slightly higher success rates in patients with initially lower bodyweight. A previous study showing that a BMI of more than 27.5 kg/m<sup>2</sup> was associated with poor results in a second look arthroscopy after mesenchymal stem cell implantation for cartilage repair<sup>36</sup> may emphasize the result of this analysis, yet there was no association found between the preoperative BMI and the clinical outcome, therefore the results regarding the association between BMI and structural and clinical outcome remain inconclusive and need to be further evaluated in future studies with a longer follow-up.

The arthroscopy revealed a slightly wider range of cartilage lesion sizes than measured preoperatively on MR images. Yet, the discrepancy between intraoperative lesion measurements and lesions size measured on preoperative MR images has been noted and discussed previously<sup>37</sup>.

This study has limitations. In this first prospective analysis, the number of patients with complete assessment of the clinical outcome and radiological data at baseline and after 2.7 years was relatively small, since this is a fairly new surgical technique. As a consequence, and also due to the paucity of previously published data on interactions between cartilage integrity and subchondral bone maturation after MACI and other repair techniques, analyses in this study were primarily performed following an exploratory approach. Therefore, multiple comparisons and correlations were assessed, and many significant findings showed only moderate correlations. Furthermore, as to be expected with exploratory approaches, multiple testing may have generated significant results. Therefore, larger study cohorts with a longer study period are needed in order to validate our findings. Furthermore, no histological analysis of the cartilage repair tissue or of the trabecular bone was provided in our study. Since there is no reference database for cartilage T2 values yet, the contralateral knee was used as an internal reference for each individual patient. Moreover, the

maximum TE could be increased and in order to increase the sensitivity of the sequence for T2 changes.

In summary, spongiosa-augmented MACI for treatment of osteochondral defects at the knee showed promising results in a mid-term follow-up 2.7 years after surgery, with the best results after osteochondral repair at the medial and lateral femur condyle. Cartilage repair tissue after spongiosa-augmented MACI, as assessed with quantitative MRI, correlates with the quality of the underlying trabecular bone. Moreover, degree of degeneration of the knee joint correlated with the quantitative parameters describing the subchondral bone. These results suggest, that cartilage and subchondral bone need to be considered as an osteochondral unit with synergistic function, particularly in the context of osteochondral repair.

#### Author contributions

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#### Competing interest statement

None of the authors have any financial or other interests related to the manuscript submitted to Osteoarthritis and Cartilage that might constitute a potential conflict of interest.

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#### Supplementary data

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#### References

- Chundru R, Baum T, Nardo L, Nevitt MC, Lynch J, McCulloch CE, et al. Focal knee lesions in knee pairs of asymptomatic and symptomatic subjects with OA risk factors—data from the Osteoarthritis Initiative. *Eur J Radiol* 2013;82:e367–73.
- Gudas R, Kalesinskas RJ, Kimtys V, Stankevicius E, Toliusis V, Bernotavicius G, et al. A prospective randomized clinical study of mosaic osteochondral autologous transplantation versus microfracture for the treatment of osteochondral defects in the knee joint in young athletes. *Arthroscopy* 2005;21:1066–75.
- Hangody L, Vasarhelyi G, Hangody LR, Sukosd Z, Tibay G, Bartha L, et al. Autologous osteochondral grafting—technique and long-term results. *Injury* 2008;39(Suppl 1):S32–9.
- Steadman JR, Rodkey WG, Briggs KK, Rodrigo JJ. The microfracture technic in the management of complete cartilage defects in the knee joint. *Orthopade* 1999;28:26–32.
- Reddy S, Pedowitz DI, Parekh SG, Sennett BJ, Okereke E. The morbidity associated with osteochondral harvest from asymptomatic knees for the treatment of osteochondral lesions of the talus. *Am J Sports Med* 2007;35:80–5.
- Behrens P, Bitter T, Kurz B, Russlies M. Matrix-associated autologous chondrocyte transplantation/implantation (MACT/MACI)—5-year follow-up. *Knee* 2006;13:194–202.
- Wondrasch B, Zak L, Welsch GH, Marlovits S. Effect of accelerated weightbearing after matrix-associated autologous chondrocyte implantation on the femoral condyle on radiographic and clinical outcome after 2 years: a prospective, randomized controlled pilot study. *Am J Sports Med* 2009;37(Suppl 1):88S–96S.
- Genovese E, Ronga M, Angeretti MG, Novario R, Leonardi A, Albrizio M, et al. Matrix-induced autologous chondrocyte implantation of the knee: mid-term and long-term follow-up by MR arthrography. *Skeletal Radiol* 2011;40:47–56.
- Gobbi A, Kon E, Berruto M, Filardo G, Delcogliano M, Boldrini L, et al. Patellofemoral full-thickness chondral defects treated with second-generation autologous chondrocyte implantation: results at 5 years' follow-up. *Am J Sports Med* 2009;37:1083–92.
- Kon E, Di Martino A, Filardo G, Tetta C, Busacca M, Iacono F, et al. Second-generation autologous chondrocyte transplantation: MRI findings and clinical correlations at a minimum 5-year follow-up. *Eur J Radiol* 2011;79:382–8.
- Saris DB, Vanlauwe J, Victor J, Almqvist KF, Verdonk R, Bellemans J, et al. Treatment of symptomatic cartilage defects of the knee: characterized chondrocyte implantation results in better clinical outcome at 36 months in a randomized trial compared to microfracture. *Am J Sports Med* 2009;37(Suppl 1):10S–9S.
- Siebold R, Suezer F, Schmitt B, Trattig S, Essig M. Good clinical and MRI outcome after arthroscopic autologous chondrocyte implantation for cartilage repair in the knee. *Knee Surg Sports Traumatol Arthrosc* 2018;26:831–9.
- Salzmann GM, Erdle B, Porichis S, Uhl M, Ghanem N, Schmal H, et al. Long-term T2 and qualitative MRI morphology after first-generation knee autologous chondrocyte implantation: cartilage ultrastructure is not correlated to clinical or qualitative MRI outcome. *Am J Sports Med* 2014;42:1832–40.
- Niethammer TR, Safi E, Ficklscherer A, Horng A, Feist M, Feist-Pagenstert I, et al. Graft maturation of autologous chondrocyte implantation: magnetic resonance investigation with T2 mapping. *Am J Sports Med* 2014;42:2199–204.
- Liess C, Lusse S, Karger N, Heller M, Gluer CC. Detection of changes in cartilage water content using MRI T2-mapping in vivo. *Osteoarthritis Cartilage* 2002;10:907–13.
- Valderrabano V, Miska M, Leumann A, Wiewiorski M. Reconstruction of osteochondral lesions of the talus with autologous spongiosa grafts and autologous matrix-induced chondrogenesis. *Am J Sports Med* 2013;41:519–27.
- Jungmann PM, Brucker PU, Baum T, Link TM, Foerschner F, Minzlaff P, et al. Bilateral cartilage T2 mapping 9 years after Mega-OATS implantation at the knee: a quantitative 3T MRI study. *Osteoarthritis Cartilage* 2015;23:2119–28.
- Ebert JR, Robertson WB, Woodhouse J, Fallon M, Zheng MH, Ackland T, et al. Clinical and magnetic resonance imaging-based outcomes to 5 years after matrix-induced autologous chondrocyte implantation to address articular cartilage defects in the knee. *Am J Sports Med* 2011;39:753–63.
- Peterfy CG, Schneider E, Nevitt M. The osteoarthritis initiative: report on the design rationale for the magnetic resonance



- imaging protocol for the knee. *Osteoarthritis Cartilage* 2008;16:1433–41.
20. Mamisch TC, Bittersohl B, Hughes T, Kim YJ, Welsch GH, Dudda M, *et al.* Magnetic resonance imaging of the hip at 3 Tesla: clinical value in femoroacetabular impingement of the hip and current concepts. *Semin Musculoskelet Radiol* 2008;12: 212–22.
  21. Peterfy CG, Guermazi A, Zaim S, Tirman PF, Miaux Y, White D, *et al.* Whole-organ magnetic resonance imaging score (WORMS) of the knee in osteoarthritis. *Osteoarthritis Cartilage* 2004;12:177–90.
  22. Baum T, Joseph GB, Arulanandan A, Nardo L, Virayavanich W, Carballido-Gamio J, *et al.* Association of magnetic resonance imaging-based knee cartilage T2 measurements and focal knee lesions with knee pain: data from the Osteoarthritis Initiative. *Arthritis Care Res (Hoboken)* 2012;64:248–55.
  23. Baum T, Sauerschnig M, Penzel J, Jungmann PM, Waldt S, Rummeny EJ, *et al.* Early changes of trabecular bone structure in asymptomatic subjects with knee malalignment. *J Comput Assist Tomogr* 2014;38:137–41.
  24. Kumar D, Schooler J, Zuo J, McCulloch CE, Nardo L, Link TM, *et al.* Trabecular bone structure and spatial differences in articular cartilage MR relaxation times in individuals with posterior horn medial meniscal tears. *Osteoarthritis Cartilage* 2013;21:86–93.
  25. Bolbos RI, Zuo J, Banerjee S, Link TM, Ma CB, Li X, *et al.* Relationship between trabecular bone structure and articular cartilage morphology and relaxation times in early OA of the knee joint using parallel MRI at 3 T. *Osteoarthritis Cartilage* 2008;16:1150–9.
  26. Blumenkrantz G, Lindsey CT, Dunn TC, Jin H, Ries MD, Link TM, *et al.* A pilot, two-year longitudinal study of the interrelationship between trabecular bone and articular cartilage in the osteoarthritic knee. *Osteoarthritis Cartilage* 2004;12: 997–1005.
  27. Welsch GH, Mamisch TC, Zak L, Blanke M, Olk A, Marlovits S, *et al.* Evaluation of cartilage repair tissue after matrix-associated autologous chondrocyte transplantation using a hyaluronic-based or a collagen-based scaffold with morphological MOCART scoring and biochemical T2 mapping: preliminary results. *Am J Sports Med* 2010;38:934–42.
  28. Domayer SE, Kutscha-Lissberg F, Welsch G, Dorotka R, Nehrer S, Gabler C, *et al.* T2 mapping in the knee after microfracture at 3.0 T: correlation of global T2 values and clinical outcome - preliminary results. *Osteoarthritis Cartilage* 2008;16:903–8.
  29. Roos EM, Roos HP, Lohmander LS, Ekdahl C, Beynon BD. Knee injury and osteoarthritis outcome score (KOOS)-development of a self-administered outcome measure. *J Orthop Sports Phys Ther* 1998;28:88–96.
  30. Gluer CC, Blake G, Lu Y, Blunt BA, Jergas M, Genant HK. Accurate assessment of precision errors: how to measure the reproducibility of bone densitometry techniques. *Osteoporos Int* 1995;5:262–70.
  31. Salzmänn GM, Niemeyer P, Steinwachs M, Kreuz PC, Sudkamp NP, Mayr HO. Cartilage repair approach and treatment characteristics across the knee joint: a European survey. *Arch Orthop Trauma Surg* 2011;131:283–91.
  32. Basad E, Wissing FR, Fehrenbach P, Rickert M, Steinmeyer J, Ishaque B. Matrix-induced autologous chondrocyte implantation (MACI) in the knee: clinical outcomes and challenges. *Knee Surg Sports Traumatol Arthrosc* 2015;23:3729–35.
  33. Kazakia GJ, Kuo D, Schooler J, Siddiqui S, Shanbhag S, Bernstein G, *et al.* Bone and cartilage demonstrate changes localized to bone marrow edema-like lesions within osteoarthritic knees. *Osteoarthritis Cartilage* 2013;21:94–101.
  34. Malinin T, Ouellette EA. Articular cartilage nutrition is mediated by subchondral bone: a long-term autograft study in baboons. *Osteoarthritis Cartilage* 2000;8:483–91.
  35. Joseph GB, Baum T, Alizai H, Carballido-Gamio J, Nardo L, Virayavanich W, *et al.* Baseline mean and heterogeneity of MR cartilage T2 are associated with morphologic degeneration of cartilage, meniscus, and bone marrow over 3 years-data from the Osteoarthritis Initiative. *Osteoarthritis Cartilage* 2012;20: 727–35.
  36. Koh YG, Choi YJ, Kwon OR, Kim YS. Second-look arthroscopic evaluation of cartilage lesions after mesenchymal stem cell implantation in osteoarthritic knees. *Am J Sports Med* 2014;42:1628–37.
  37. Gomoll AH, Yoshioka H, Watanabe A, Dunn JC, Minas T. Pre-operative measurement of cartilage defects by MRI underestimates lesion size. *Cartilage* 2011;2:389–93.