Osteoarthritis and Cartilage 27 (2019) 90–98

Osteoarthritis and Cartilage

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

A.S. Gersing † *, G. Feuerriegel †, C. Holwein ‡ §, J. Suchowierski ‡, D.C. Karampinos †, B. Haller #, T. Baum ||, B.J. Schwaiger †, J.S. Kirschke ||, E.J. Rummeny †, A.B. Imhoff ‡, K. Woertler †, P.M. Jungmann † ¶

† *Department of Radiology, Klinikum Rechts der Isar, Technical University of Munich, Munich, Germany*
‡ *Department of Orthopaedic Sports Medicine, Klinikum Rechts der Isar, Technical University of Munich, Munich, Germany*
§ *Department of Trauma and Orthopaedic Surgery, BG Unfallklinik Murnau, Murnau, Germany*
|| *Department of Neuroradiology, Klinikum Rechts der Isar, Technical University of Munich, Munich, Germany*
¶ *Department of Neuroradiology, University Hospital Zurich, University of Zurich, Switzerland*
# *Institute of Medical Informatics, Statistics and Epidemiology, Technical University of Munich, Munich, Germany*

ARTICLE INFO

Article history:
Received 30 December 2017
Accepted 30 August 2018

Keywords:
Magnetic resonance imaging
Knee joint
Articular cartilage
Osteochondral defect
Matrix-associated autologous chondrocyte implantation

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 ± 7.8y; 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-precision 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 ± 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 ± 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.

Introduction

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

* Address correspondence and reprint requests to: A. S. Gersing, Department of Radiology, Technical University of Munich, Ismaninger Strasse 22, 81675 Munich, Germany. Tel: 49-(0)89-4140-5428; Fax: 49-(0)89-4140-4834.
E-mail addresses: alexandra.gersing@tum.de (A.S. Gersing), g.feuerriegel@gmail.com (G. Feuerriegel), christian.holwein@klinikum-gap.de (C. Holwein), joachim.suchowierski@gmail.com (J. Suchowierski), dimitrios.karampinos@tum.de (D.C. Karampinos), bernhard.haller@tum.de (B. Haller), thomas.baum@tum.de (T. Baum), bschwaiger@gmx.com (B.J. Schwaiger), jan.kirschke@tum.de (J.S. Kirschke), ernst.rummeny@tum.de (E.J. Rummeny), imhoff@tum.de (A.B. Imhoff), klaus.woertler@tum.de (K. Woertler), pia.jungmann@usz.ch (P.M. Jungmann).

https://doi.org/10.1016/j.joca.2018.08.023
1063-4584/

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[6,8–11]; few studies have evaluated outcome of patients that had undergone MACI with quantitative MR imaging[12–14]. 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[15]. 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[16]. 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, unicompartmental 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 ± 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 ± 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.

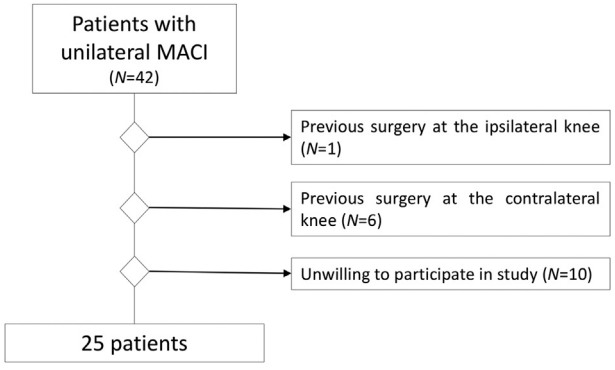

Fig. 1. Flow chart illustrating patient selection.

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 (±standard deviation) between the ipsilateral knee and the contralateral knee of cartilage T2 averaged over all compartments was 1.7 ± 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[17]. 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[18]. 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 I. 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[19]. 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

Table I
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^2)	0.4 × 0.4	0.4 × 0.4	0.4 × 0.4	0.2 × 0.2	0.4x0.4
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[20], 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[21,22]. 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[23]. 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[23–26]. 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[24–26]. Histomorphometric parameters were then calculated using a mean intercept length method and the following values were calculated from the sequence[23]: 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[27,28]. 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[15]. 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)[29] was used assessing knee pain, symptoms other than pain, activities of daily living (ADL), sport and recreation and related quality of life.

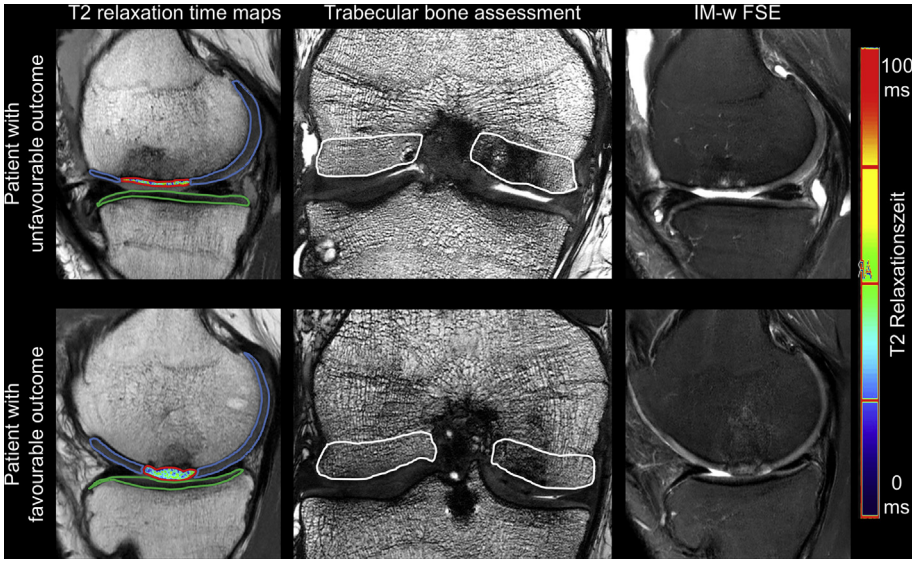

Fig. 2. Exemplary MR images and T2-maps of a 24-year-old male patient with high subchondral trabecular thickness at the osteochondral repair site (upper row) compared to the contralateral knee and a 22-year old male patient with low trabecular thickness at the osteochondral repair site (lower row) compared 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 ± 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, WORMS at follow-up, WORMS progression (=WORMS at follow-up − WORMS 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 WORMS >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 WORMS 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 WORMS 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[30]. 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 cm^2 (range 1.5–7.0 cm^2). All patients ($n = 25$) presented an osteochondral lesion and the average depth of the bone defect was 3.47 ± 1.98 mm and the average length of the bone lesion was 13.42 ± 6.68 mm. The intraoperatively measured cartilage lesion size was 3.8 cm^2 (range 1.8–8.8 cm^2). The average BMI was 25.8 ± 4.5 kg/m^2. The follow-up time averaged over all patients was 2.7 ± 0.3 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 ± 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 Th.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	**0.03**
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	**0.01**
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	**0.47**	0.21	−0.07	0.42	0.05	**0.36**	0.20	0.31
	P-value*	**0.032**	0.38	0.76	0.06	0.82	**0.031**	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	**0.44**	−0.22	−0.25
	P-value*	0.36	0.85	0.60	0.63	0.84	**0.041**	0.33	0.29

*P-values <0.05 are in bold.

Morphological outcome

The average MOCART score was 67.7 ± 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[31].

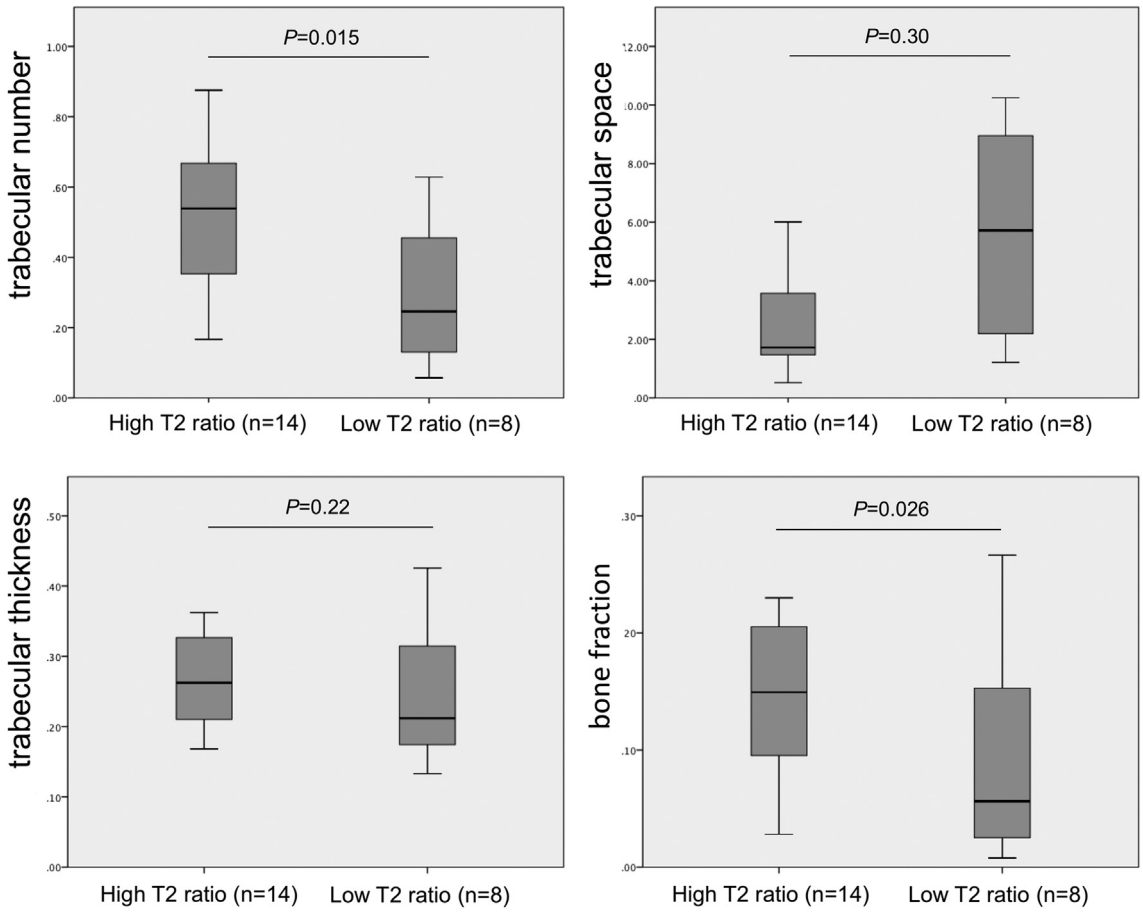

Fig. 3. Comparison of the average ratio of trabecular parameters at the osteochondral repair site (ipsilateral/contralateral knee) between patients with a high cartilage T2 ratio (ipsilateral cartilage T2/contralateral cartilage T2, $n = 14$) and patients with low cartilage T2 ratio ($n = 11$) in the 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	**0.48**	−0.22	**0.57**	**0.48**
	P-value*	**0.031**	0.34	**0.007**	**0.034**
Meniscus	Correlation coefficient	**0.65**	−0.10	−0.04	0.03
	P-value*	**0.001**	0.66	0.85	0.90
Cartilage	Correlation coefficient	0.42	−0.06	**0.60**	0.42
	P-value*	0.06	0.81	**0.004**	0.06
BME	Correlation coefficient	−0.36	−0.274	**0.49**	**0.49**
	P-value*	0.11	0.23	**0.031**	**0.022**
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	**−0.46**	0.38	**−0.49**	−0.41
	P-value*	**0.03**	0.08	**0.02**	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[18,32]. 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[33]. 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[34]. 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[33,35]. 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^2 was associated with poor results in a second look arthroscopy after mesenchymal stem cell implantation for cartilage repair[36] 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[37].

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

Alexandra S. Gersing, MD (alexandra.gersing@tum.de) and Pia M Jungmann, MD (pia.jungmann@usz.ch) take responsibility for the integrity of the work as a whole, from inception to finished article.

Conception and design of the study: Gersing, Feuerriegel, Holwein, Suchowierski, Karampinos, Baum, Schwaiger, Kirschke, Rummeny, Imhoff, Woertler, Jungmann.

Acquisition of data: Gersing, Feuerriegel, Holwein, Suchowierski, Karampinos, Jungmann.

Analysis and interpretation of data: Gersing, Feuerriegel, Holwein, Suchowierski, Karampinos, Haller, Baum, Schwaiger, Kirschke, Rummeny, Imhoff, Woertler, Jungmann.

Drafting of article or revising it critically for important intellectual content: Gersing, Feuerriegel, Holwein, Suchowierski, Karampinos, Haller, Baum, Schwaiger, Kirschke, Rummeny, Imhoff, Woertler, Jungmann.

Final approval of the version of the article to be published: Gersing, Feuerriegel, Holwein, Suchowierski, Karampinos, Haller, Baum, Schwaiger, Kirschke, Rummeny, Imhoff, Woertler, Jungmann.

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.

Acknowledgments

This study was funded by the B. Braun-Stiftung.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.joca.2018.08.023.

References

1. 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.
2. 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.
3. 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.
4. 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.
5. 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.
6. 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.
7. 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.
8. 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.
9. 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.
10. 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.
11. 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.
12. Siebold R, Suezer F, Schmitt B, Trattnig 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.
13. 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.
14. 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.
15. 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.
16. 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.
17. 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.
18. 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.
19. 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 Muscoskel 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, Beynnon 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. Salzmann 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. Preoperative measurement of cartilage defects by MRI underestimates lesion size. Cartilage 2011;2:389–93.