Evaluation of native hyaline cartilage and repair tissue after two cartilage repair surgery techniques with $^{23}$Na MR imaging at 7 T: initial experience

Š. Zbýn††, D. Stelzeneder †, G.H. Welsch †§, L.L. Negrin ‡, V. Juras ⦿, M.E. Mayerhoefer †, P. Szomolanyi ⦿§, W. Bogner †, S.E. Domayer ⦿, M. Weber †, S. Trattnig †

*MR Centre-Highfield MR, Department of Radiology, Medical University of Vienna/Vienna General Hospital, Vienna, Austria
†Department of Orthopedic Surgery, Medical University of Vienna/Vienna General Hospital, Vienna, Austria
‡Department of Trauma Surgery, University Hospital of Erlangen, Erlangen, Germany
§Department of Trauma Surgery, Medical University of Vienna/Vienna General Hospital, Vienna, Austria
#Department of Imaging Methods, Institute of Measurement Science, Slovak Academy of Sciences, Bratislava, Slovakia

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SUMMARY

Objective: To compare the sodium normalized mean signal intensity (NMSI) values between patients after bone marrow stimulation (BMS) and matrix-associated autologous chondrocyte transplantation (MACT) cartilage repair procedures.

Methods: Nine BMS and nine MACT patients were included. Each BMS patient was matched with one MACT patient according to age [BMS 36.7 ± 10.7 (mean ± standard deviation) years; MACT 36.9 ± 10.0 years], postoperative interval [BMS 33.5 ± 25.3 months; MACT 33.2 ± 25.7 months], and defect location. All magnetic resonance imaging (MRI) measurements were performed on a 7 T system. Proton images served for morphological evaluation of repair tissue using the magnetic resonance observation of cartilage repair tissue (MOCART) scoring system. Sodium NMSI values in the repair area and morphologically normal cartilage were calculated. Clinical outcome was assessed right after MRI. Analysis of covariance, t-tests, and Pearson correlation coefficients were evaluated.

Results: Sodium NMSI was significantly lower in BMS ($P = 0.004$) and MACT ($P = 0.006$) repair tissue, compared to reference cartilage. Sodium NMSI was not different between the reference cartilage in MACT and BMS patients ($P = 0.664$); however, it was significantly higher in MACT than in BMS repair tissue ($P = 0.028$). Better clinical outcome was observed in BMS than in MACT patients. There was no difference between MOCART scores for MACT and BMS patients ($P = 0.915$). We did not observe any significant correlation between MOCART score and sodium repair tissue NMSI ($r = -0.001; P > 0.996$).

Conclusions: Our results suggest higher glycosaminoglycan (GAG) content, and therefore, repair tissue of better quality in MACT than in BMS patients. Sodium imaging might be beneficial in non-invasive evaluation of cartilage repair surgery efficacy.

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Introduction

Injuries of articular cartilage are one of the most common types of injuries in orthopedic practice. The articular cartilage of adults shows no or minimal potential for self-healing of chondral defects that exceed a critical size and such defects often progress to osteoarthritis (OA). Therefore, various treatment procedures, such as bone marrow stimulation (BMS) techniques including microfracture (MFX) and subchondral drilling, or advanced cell-based cartilage repair surgery techniques, including autologous chondrocyte implantation (ACI) and matrix-associated autologous chondrocyte transplantation (MACT), have been developed. BMS techniques produce bleeding from the subchondral bone to promote clot formation and a subsequent healing cascade forms scar tissue that fills the chondral defect. The goal of cartilage repair procedures is to restore joint function and prevent OA by providing repair tissue that has structure, composition, and biomechanical properties similar to native articular cartilage.

Articular cartilage consists of small amount of chondrocytes embedded in a large extracellular matrix which is created by water molecules, collagen fibers and proteoglycan macromolecules made of a protein core with attached glycosaminoglycan (GAG) side
The negatively charged residues of the sulfate and carboxyl groups of GAG attract positive counter-ions (such as sodium) and water molecules, and provide strong electrostatic and osmotic forces responsible for the functional and structural properties of cartilage. Therefore, a lot of effort has been made to develop methods for the non-invasive monitoring of the GAG content in native cartilage and cartilage repair tissue, such as delayed gadolinium-enhanced magnetic resonance imaging (MRI) of cartilage (dGEMRIC) or sodium imaging.

Based on the fact that GAG molecules are counterbalanced by sodium ions, sodium imaging was successfully used for the evaluation of GAG (and hence, proteoglycan) content in the cartilage of healthy humans and, recently, in patients after MACT.

Therefore, the aim of this study was to compare sodium normalized mean signal intensities (NMSIs) at 7 T, suggestive of GAG content, between native cartilage and repair tissue of patients after BMS and MACT repair procedures.

Materials and methods

Patients

This cross-sectional study was approved by the Institutional Review Board and written informed consent was obtained from all patients prior to enrollment in the study. From our database of about 135 follow-up patients, 15 BMS patients (six women, nine men; mean age, 38.1 ± 14.2 years; age range, 21.4–67.3 years; three subchondral drillings, and 12 MFX patients), and 29 MACT patients (10 women, 19 men; mean age, 35.8 ± 11.8 years; age range, 22.4–60.6 years) agreed to undergo additional sodium MRI at 7 T between July 2009 and September 2010. From this patient cohort, the best matching MACT partner was assigned to each BMS patient. Only pairs of patients with age difference of less than 3.5 years, postoperative interval difference of less than 3 months and similar defect location were included in this study. Matching criteria passed nine BMS patients (two Pridie drilling, seven MFX patients; four women, five men; mean age, 36.7 ± 10.7 years (± standard deviation) years; age range, 21.4–57.7 years; mean postoperative interval, 33.5 ± 25.3 months), and nine MACT patients (three women, six men; mean age, 36.9 ± 10.0 years; age range, 24.6–56.0 years; mean postoperative interval, 33.2 ± 25.7 months). The difference between matched patients ranged between 0.1 and 3.1 years for the age and 0–2.8 months for the postoperative interval. The repair tissue was situated at the medial femoral condyle (five BMS and five MACT patients), lateral femoral condyle (four BMS and two MACT patients), or the trochlea region (two MACT patients). The mean body mass index (BMI) was 25.1 ± 2.4 in BMS and 23.8 ± 2.4 in MACT patients.

Only patients with single symptomatic full-thickness cartilage defect caused either by trauma or pre-existing osteochondritis dissecans were included in the study. Exclusion criteria were advanced OA, meniscal tear, knee ligament injuries, metallic implants, and knee joint instability that were evaluated with standard clinical testing. Patients with advanced OA were defined as patients with cartilage defect of grade 3 or 4 according to the International Cartilage Repair Society classification. All evaluated criteria were assessed preoperatively with radiographs and conventional MRI of the knee joint, and verified and documented at the time of cartilage repair surgery. The mean defect size was 2.5 ± 0.8 cm² (range, 1.5–3.6 cm²) in BMS and 5.0 ± 1.3 cm² (range, 3.2–7.3 cm²) in MACT patients.

Both employed BMS techniques, subchondral drilling, and MFX, are marrow stimulating techniques which rely on the same biological principles and therefore are producing comparable type of repair tissue. To our best knowledge, no systematic comparison of the cartilage repair outcome between these techniques was done yet. The following three-dimensional scaffolds, seeded with autologous chondrocytes, were used in MACT treatment: Hyalograft C (Fidia Advanced Biopolymers, Abano Terme, Italy); CaReS (Arthro Kinetics, Esslingen, Germany); or BioCart II (ProChon Biotech, Woburn, MA). All MACT and BMS patients followed the same established protocols for the rehabilitation after repair surgery on the femoral condyle or trochlea.

Clinical outcome

The International Knee Documentation Committee (IKDC) Subjective Knee Form and the Modified Cincinnati Knee Rating were used to evaluate clinical outcome after cartilage repair surgery. The IKDC Subjective Knee Form measures symptoms, function, and sports activity in patients with pathologies in the knee joint. Higher scores (maximum 100 points) are denoting greater levels of function and lower knee symptoms.

The Modified Cincinnati Knee Rating evaluates limitations in daily life, ranging from severe limitations to unlimited full functionality (maximum 10 points) and gives an overview of managing daily activities.

MRI

All MRI measurements were acquired with an optimized sodium MRI at 7 T whole body system (Magnetom, Siemens Healthcare, Erlangen, Germany) using a 28-channel knee coil (Quality Electrodynamics LLC, Cleveland, OH) and a 23Na-only circularly polarized knee coil (Stark Contrast, Erlangen, Germany). Normalization sample containing 308 mmol/L NaCl solution was fixed to the sodium coil to serve for normalization of the sodium signal.

For the morphological evaluation of cartilage repair tissue served a proton density-weighted two-dimensional turbo spin echo (2D-TSE) sequence with fat suppression in the sagittal and coronal plane, and a T1-weighted three-dimensional gradient echo (3D-GRE) sequence (Table 1). The total measurement time for morphological imaging was about 13 min.

All sodium measurements were acquired with an optimized 3D-GRE sequence. To comply with the specific absorption rate limits and minimize echo time, the excitation pulse length was 970 µs (Table 1). The readout length was 5.88 ms. The total measurement time for sodium imaging with the nominal resolution of 3.11 × 1.55 × 3.0 mm³, including flip angle calibration, was less than 34 min.

Table 1: Parameters of proton and sodium MR sequences used in presented study

<table>
<thead>
<tr>
<th>Sequence</th>
<th>TR (echo time) (TE) (msec)</th>
<th>Flip angle (degrees)</th>
<th>Bandwidth (Hz/pixel)</th>
<th>Field of view (mm²)</th>
<th>Matrix size</th>
<th>Resolution (mm³)</th>
<th>Section thickness (ST) (mm)</th>
<th>No. of slices</th>
<th>No. of averages</th>
<th>Acquisition time (TA) (min:sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sagittal 2D-TSE</td>
<td>3.400/26</td>
<td>125</td>
<td>243</td>
<td>160 × 129</td>
<td>448 × 360</td>
<td>0.36 ± 0.36</td>
<td>3.0</td>
<td>20</td>
<td>1</td>
<td>4:20</td>
</tr>
<tr>
<td>Coronal 2D-TSE</td>
<td>2.400/24</td>
<td>122</td>
<td>243</td>
<td>160 × 159</td>
<td>448 × 377</td>
<td>0.36 ± 0.42</td>
<td>3.0</td>
<td>15</td>
<td>1</td>
<td>1:21</td>
</tr>
<tr>
<td>1H-3D-GRE</td>
<td>8.3/3.6</td>
<td>8</td>
<td>450</td>
<td>160 × 135</td>
<td>384 × 324</td>
<td>0.42 ± 0.24</td>
<td>0.45</td>
<td>224</td>
<td>1</td>
<td>6:09</td>
</tr>
<tr>
<td>23Na-3D-GRE</td>
<td>10/3.77</td>
<td>56</td>
<td>170</td>
<td>199 × 199</td>
<td>128 × 64</td>
<td>1.55 × 3.11</td>
<td>3.0</td>
<td>48</td>
<td>60</td>
<td>30:45</td>
</tr>
</tbody>
</table>
Evaluation of proton MRI

The proton images served for the morphological evaluation of cartilage and bone after repair surgery using the magnetic resonance observation of cartilage repair tissue (MOCART) scoring system\(^2\)\(^5\). A senior musculoskeletal radiologist (ST, with 15 years of experience) assigned a MOCART score to each repair site of each patient. MOCART is a reproducible grading system that has been applied to different cartilage repair techniques\(^1\)\(^9\),\(^2\)\(^5\),\(^2\)\(^6\). A point score was subscribed to each variable, with a maximum score of 100 points representing excellent morphological outcome of repair tissue, and 0 representing poor outcome.

Evaluation of sodium MRI

According to the morphological images and the intraoperative documentation, one sodium image containing the largest amount of cartilage repair tissue was selected and used for evaluation. All regions-of-interest (ROI) were defined by a senior musculoskeletal radiologist (ST, with 15 years of experience) in consensus with an orthopedic surgeon with a special interest in musculoskeletal MRI (GHW, with 10 years of experience). For ROI analyses, sodium image was rescaled to resolution of 2D-TSE images and overlaid with corresponding 2D-TSE image in picture editing tool (Adobe Photoshop CS2, version 9.0). The ROIs defined on 2D-TSE image were transferred to sodium image and evaluated using the JiveX DICOM Viewer (JiveX 4.3, VISUS Technology Transfer GmbH, Bochum, Germany). To avoid partial volume artifacts between repair tissue and surrounding native cartilage, the border region between repair and native tissue was excluded from the repair ROIs. The reference ROI was defined in morphologically normal-appearing cartilage that was exposed to similar weight-bearing conditions as the corresponding repair tissue. A minimum distance of 8 mm was maintained between the repair and reference ROIs. Both, repair and reference ROIs were defined in accordance with the corresponding proton morphological images and the intraoperative documentation. The sizes of both ROIs were kept as similar as possible, with a mean area of 24.6 \(\pm\) 7.6 mm\(^2\) (range, 12.1–38.7 mm\(^2\)) in the reference ROIs, and a mean area of 26.1 \(\pm\) 7.4 mm\(^2\) (range, 15.5–38.7 mm\(^2\)) in the repair ROIs (Figs. 1 and 2).

The signal-to-noise ratio (SNR) was calculated by dividing the mean signal intensity from the reference or repair ROI by the standard deviation of the signal intensity in a ROI defined in the signal-free area of the same image. The NMSI values were calculated by multiplying the mean sodium signal from the reference or repair ROIs with the normalization factor. This factor is the ratio of the highest sodium signal in the normalization sample, over all patients, to the sodium signal in the normalization sample of the patient under consideration. For better comparability of cartilage repair techniques, we calculated the sodium repair-to-reference signal intensity ratio, characterized as the ratio between the signal intensity from the repair ROI and the corresponding reference ROI.

To evaluate reproducibility of sodium measurements, 20-year-old woman after MACT on medial femoral condyle was measured twice. When comparing the NMSI values between the measurements, the mean coefficient of variation was 2.25\% (range 0.32–3.81\%) and the mean difference 3.12\% (range 0.46–5.25\%).

Fig. 1. Sagittal proton density-weighted 2D-TSE MR image with fat suppression (left); sagittal sodium 3D-GRE image (middle); and color-coded sagittal sodium 3D-GRE image (right) in a 43-year-old woman obtained 42 months after an MFX procedure. Cartilage repair tissue is situated between the two arrows. Red contours in the middle image represent the ROI analysis of repair tissue (right contour) and reference cartilage (left contour). Please note that repair tissue voxels situated closest to the repair tissue-native cartilage interface are not included into the ROI evaluations. Color scale represents the sodium signal intensity values.

Fig. 2. Sagittal proton density-weighted 2D-TSE MR image with fat suppression (left); sagittal sodium 3D-GRE image (middle); and color-coded sagittal sodium 3D-GRE image (right) in a 35-year-old woman obtained 50.6 months after MACT surgery. Cartilage repair tissue is situated between the two arrows. Red contours in the middle image represent the ROI analysis of repair tissue (left contour) and reference cartilage (right contour). Please note that repair tissue voxels situated closest to the repair tissue-native cartilage interface are not included into the ROI evaluations. Color scale represents the sodium signal intensity values.
Sodium MRI results

The mean sodium SNR value in the BMS patients was 20.6 ± 4.8 (range, 13.2–26.1) in reference cartilage and 12.0 ± 2.5 (range, 7.3–15.1) in repair tissue. The patients after the MACT procedure showed mean sodium SNR of 21.8 ± 3.0 (range, 19.4–28.2) in reference cartilage and 17.0 ± 3.2 (range, 11.3–23.7) in repair tissue. The mean sodium NMSI value was 279 ± 47 (range, 205–336) in reference cartilage and 164 ± 31 (range, 127–216) in repair tissue of BMS patients (Fig. 3). The MACT patients revealed mean sodium NMSI of 270 ± 36 (range, 227–322) in reference cartilage and 210 ± 36 (range, 165–277) in repair tissue (Fig. 3). The mean sodium NMSI values from repair tissue were lower compared to corresponding values from reference cartilage for each patient and for both types of treatment techniques (Fig. 4). In each matched pair of patients, the sodium repair-to-reference signal intensity ratio was higher in patients after MACT, compared to BMS patients (Fig. 5).

A two-way repeated measures ANCOVA did not reveal significant influence of surgery (P = 0.124), age (P = 0.995), follow-up interval (P = 0.059), BMI (P = 0.248) or tissue type (P = 0.117) on sodium NMSI values. However, the ANCOVA showed statistically significant influence of surgery type (P = 0.026), but no significant influence of age (P = 0.201), follow-up interval (P = 0.294) or BMI (P = 0.624) on the difference in NMSI values between reference cartilage and repair tissue. A post hoc paired samples t-test revealed significantly lower sodium NMSI in repair tissue compared to corresponding reference cartilage in the BMS patients (P = 0.004), as well as in the MACT subjects (P = 0.006) (Fig. 3). There was no significant difference in the sodium NMSI of reference cartilage when comparing patients after MACT and BMS treatment with paired t-test (P = 0.664) (Fig. 3). Significantly higher sodium NMSI was observed in repair tissue after the MACT procedure compared to the BMS techniques (P = 0.028) using a paired t-test (Fig. 3). Similarly, a paired t-test showed significantly higher sodium repair-to-reference signal intensity ratio in MACT patients when compared to BMS patients (P = 0.003).

Clinical outcome and morphological evaluations

The mean IKDC Subjective Knee Form score was 78.8 [standard error of the mean (SE) = 11.2; 95% confidence interval (CI) = 47.7 to 109.9] for five BMS and 64.1 (SE = 9.6; CI = 37.3 to 90.9) for five MACT patients. The mean Cincinnati Knee Rating was 8.0 (SE = 0.7; CI = 6.0 to 10.0) for five BMS and 6.0 (SE = 1.2; CI = 2.7 to 9.3) for five MACT patients. No statistically significant difference was observed between BMS and MACT patients when comparing the results of IKDC Subjective Knee Form (P = 0.408) or the Modified Cincinnati Knee Rating (P = 0.275) using paired t-test. However, BMS patients had in average 14.7 points more (CI = −29.5 to 58.9) in IKDC Subjective Knee Form scoring and two points more (CI = −2.4 to 6.4) in Cincinnati Knee Rating (Fig. 6). These differences were considered as clinically relevant. No significant difference in BMI values was observed between BMS and MACT patients using paired t-test (P = 0.076).

The mean MOCART score was 75.0 ± 16.6 points (range, 50–100 points) in BMS and 73.9 ± 16.7 points (range, 55–95 points) in MACT patients. A paired t-test did not show a significant difference between the MOCART scores of BMS and MACT patients (P = 0.915).

Linear associations

There was no significant correlation between clinical outcome scores and sodium NMSI in repair tissue (IKDC scoring: r = −0.382; P = 0.276; CI = −0.815 to 0.326; Cincinnati rating: r = −0.521; P = 0.123; CI = −0.866 to 0.162). Medium association was found between clinical outcome scores and sodium repair-to-reference signal intensity ratio (IKDC scoring: r = −0.502; P = 0.139; CI = −0.860 to 0.187; Cincinnati rating: r = −0.549; P = 0.100; CI = −0.876 to 0.123) [Fig. 7(a and b)]. No linear association between the MOCART score and sodium NMSI from repair tissue (r = −0.001; P = 0.996; CI = −0.468 to 0.466), or between the MOCART score and the sodium repair-to-reference signal intensity ratio (r = 0.232; P = 0.354; CI = −0.263 to 0.631) was observed [Fig. 7(c)]. Low correlation was observed between sodium NMSI in

Statistical analyses

To compare the sodium NMSI between the repair and the reference regions, by taking different type of surgery, as well as age and follow-up interval into account, an analysis of covariance was performed by using a two-way repeated measures analysis of covariance (ANCOVA) with four covariates (surgery, age, follow-up interval and BMI), and one within-subject effect (tissue type). All post hoc NMSI comparisons were achieved with a two-tailed paired t-test and were corrected for multiple test errors according to Bonferroni–Holm. A Pearson correlation coefficient (r) was determined to evaluate the associations between results of sodium imaging and MOCART scoring, and between sodium imaging and patient’s age and postoperative interval. The strength of association was classified as none correlation (r < 0.3), low (0.3 < r < 0.5), moderate (0.5 < r < 0.7), or strong (r > 0.7). All statistical evaluations were performed using the SPSS software (SPSS 15.0 for Windows; SPSS Inc., Chicago, IL) and a P-value equal to or less than 0.05 was considered statistically significant.
repair tissue and postoperative follow-up interval \((r = -0.359; P = 0.143; CI = -0.707 \text{ to } 0.130)\), and no association was observed between sodium repair-to-reference signal intensity ratio and postoperative follow-up interval \((r = -0.096; P = 0.704; CI = -0.539 \text{ to } 0.388)\) [Fig. 7(d)].

**Discussion**

The aim of this study was to compare the sodium NMSI values from native cartilage and repair tissue between the patients after MACT and BMS cartilage repair procedures. Significantly lower sodium NMSI values in repair tissue than in native cartilage found in MACT and BMS patients indicate lower GAG content in repair tissue than in native cartilage. Higher sodium NMSI values observed in MACT patients suggest higher GAG content, and therefore, repair tissue of better quality than the repair tissue after BMS techniques. However, higher sodium NMSI values in repair tissue did not result in better clinical outcome of the MACT patients in this study.

Fig. 4. Graphs comparing the mean sodium NMSI values between the reference cartilage and the repair tissue of patients after BMS (a) and MACT (b) repair techniques. Each pair of bars represents one patient. The patients are ordered according to the follow-up interval between surgery and sodium MR imaging plotted on the x-axis. Note the matching follow-up interval between corresponding BMS and MACT patients.

Relatively simple and low-cost BMS techniques are limited to the treatment of smaller, isolated chondral defects \((1-3 \text{ cm}^2)\)\(^{27}\). More sophisticated cartilage repair surgery techniques, such as MACT, allow treatment of larger \((\text{up to } 8 \text{ cm}^2)\) defects\(^{28}\). MACT relies on three-dimensional biodegradable scaffolds that produce a cartilage-like repair tissue\(^{7,29,30}\). Conversely, BMS techniques fill the defect mostly with fibrocartilaginous repair tissue, which lacks the structural, biomechanical, and biochemical properties of native hyaline cartilage\(^{4,22,31,32}\). The GAG content comprises 3--10% of the extracellular matrix in native cartilage\(^9\) and provides cartilage with functional and structural properties\(^{11}\). Under ideal conditions, repair tissue produced by the cartilage repair techniques should, over time, develop and maintain GAG content similar to hyaline cartilage. Despite the mentioned advantages of MACT, its role as an alternative to BMS techniques is not thoroughly defined yet\(^{33}\). Therefore, the ability to track changes in native cartilage and repair tissue non-invasively is crucial for understanding of the impact of therapeutic procedures.

Using sodium MRI at 7 T in patients after different cartilage repair surgeries, we found significantly lower sodium NMSI in repair tissue after BMS and MACT treatment compared to corresponding reference native cartilage. Moreover, MACT repair tissue demonstrated significantly higher sodium NMSI than repair tissue after BMS. Sodium MRI has been validated as a quantitative method for the calculation of GAG (and hence, proteoglycan) concentration for healthy\(^{17,34}\) and trypsin-degraded cartilage\(^{15,35}\), but not for repair tissue after different cartilage repair surgeries. On the other hand, dGEMRIC has been validated as a method for measuring the GAG concentration in native cartilage and repair tissue\(^{26,37}\). Recently, Trattnig et al. demonstrated a strong correlation of sodium imaging with dGEMRIC in native and repair tissue\(^{38}\). Thus, it seems reasonable to assume that sodium imaging is indicative of GAG content, both, in native cartilage and in repair tissue. Our findings may indicate higher GAG content in MACT repair tissue compared to repair tissue after BMS techniques. Furthermore, the presented data suggest a lower GAG content in repair tissue after both techniques when compared to the native reference cartilage. Similarly, significantly lower sodium NMSI in MACT repair tissue compared to reference cartilage was reported previously\(^{39}\).

To our knowledge, there has been no prior sodium imaging study comparing the patients after different cartilage repair procedures. However, prior dGEMRIC and histological studies of cartilage repair can be compared with our findings. Varying results from previous histological studies might be explained by the limited size of tissue harvested from the whole repair tissue area,

Fig. 5. Bar plot comparing the sodium repair-to-reference signal intensity ratios between the matched pair of patients after BMS and MACT repair surgeries. Each pair of bars in the plot represents the comparison between two matched patients with different types of repair surgery. Sodium repair-to-reference signal intensity ratios are ordered according to the mean follow-up interval between surgery and sodium MR imaging of matched patients. Note that all repair-to-reference signal intensity ratios of MACT patients are higher compared to BMS patients in each matched pair of patients.
which might not be of homogeneous histological quality. Although Knutsen et al. did not observe a significant difference between histological evaluation of repair tissue produced by MFX and ACI, hyaline-like repair tissue was observed more frequently in ACI patients but without significant differences in the clinical outcome when compared to MFX patients. Conversely, the repair tissue after ACI, using characterized cell therapy, demonstrated statistically significant improvement of structural quality, and resulted in a statistically significant improvement of clinical outcome when compared to MFX. Similarly, Gudas et al. reported only fibrocartilage in biopsies from repair tissue after MFX, and other studies showed 27–75% of biopsies with hyaline-like cartilage in tissue after MACT. Since fibrocartilage demonstrates a lower GAG content compared to native hyaline cartilage, the results of our study correspond well with this data. In contrast to studies reported above, we observed better clinical outcome in BMS than in MACT patients. Thus, higher sodium NMSI values in repair tissue, suggestive of higher GAG content, did not result in better clinical outcome of the MACT patients in this study. Although GAGs strongly influence the functional properties of cartilage, it seems there is no direct relationship between the GAG content and the patient’s clinical outcome.

The dGEMRIC technique has been shown to be sensitive to the GAG content of cartilage. Previous dGEMRIC studies reported a significant difference between reference cartilage and repair tissue in MACT patients at different follow-up periods. Similar to the presented results, dGEMRIC was able to differentiate between different types of cartilage repair tissues, and found lower GAG content in repair tissue after MFX compared to MACT.

We found no linear association between the morphological MOCART score and biochemical sodium imaging. Similarly, Tins et al. found no correlation between MRI parameters and graft histological appearance.

In presented study, we found no or only low linear association between the follow-up interval and sodium values from BMS and MACT repair tissue. Similarly, no significant difference was found in a dGEMRIC study that compared two groups of MACT patients; 3–13 months and 19–42 months after repair surgery. However, histological and clinical data suggest an increase in GAG content of MACT repair tissue, over the 1-year period after repair surgery. A larger longitudinal study would be necessary to adequately evaluate GAG changes after repair surgery.

Clinical applicability of sodium imaging is limited because it requires special hardware with multinuclear capability, sodium coils and preferably very high field strength (>3 T) to provide sufficient SNR in a clinically acceptable time. The last issue may be overcome by employing projection imaging or 3D cones techniques which may allow to transfer sodium MRI from 7 T to clinical 3T systems. Other available GAG-sensitive techniques, such as dGEMRIC, T1ρ and GAG chemical exchange saturation transfer (gagCEST), have also certain limitations. Major limitation of dGEMRIC is the need of intravenous contrast agent and the time delay between its administration and the MRI. Although T1ρ is affected by GAG concentration, it is also influenced by other relaxation mechanisms, especially by dipolar interaction. The gagCEST is sensitive to patient motion and static magnetic field inhomogeneities which require postprocessing corrections. Sodium imaging can be seen as reference standard for GAG content assessment and can help evaluate specificity of new GAG-sensitive techniques.

Although measured NMSI values are proportional to GAG content, only absolute quantification of sodium content can provide information about the GAG concentration in native cartilage and repair tissue. For this calculation, it is necessary to correct sodium signal for longitudinal relaxation (T1) and for short (T2f) and long (T2s) components of the biexponential transverse relaxation decay and to use a calibration curve for assigning the signal intensity to a sodium concentration.

Mainly due to short biexponential transversal relaxation times and much lower concentration of sodium than proton nuclei in the articular cartilage, sodium images are in general acquired with low resolution in order to achieve sufficiently high SNR. Consequently, ROI evaluations of sodium images are prone to partial volume errors, which could result in overestimation of sodium signal in repair tissue. This possible source of error was overcome by excluding repair voxels situated on the interface between repaired tissue and native cartilage from the repair ROIs. Also voxels containing both, cartilage and synovial fluid could result in
overestimated sodium signal. The contribution of synovial fluid to sodium signal was minimized using repetition time (TR) of 10 ms, which resulted in heavily T1-weighted images. Another limitation of presented study is unknown sodium relaxation times in the repair tissue. Time demanding relaxation measurements were not added to protocol and sodium relaxations times in repair tissue have not yet been published. An estimate of changes in relaxation times between repair tissue and native cartilage may provide in vitro relaxation study\textsuperscript{57}. In this study, GAG depletion resulted in prolongation of T1 and T2s times and shortening of T2f time. This change could decrease presented NMSI values in addition to their decrease due to GAG loss. When correcting repair NMSI for the decrease caused by the relaxation times change, we observed again significantly higher sodium in the MACT repair tissue than in the BMS repair tissue (\(P = 0.043\)) using a paired t-test. On the other hand, changes in relaxation times make presented T1-weighted sodium imaging more sensitive to small changes in GAG content\textsuperscript{38} and therefore more attractive for clinical practice. Another limitation of our preliminary study is the low number of patients. Only nine pairs were satisfactorily matched for age, follow-up interval, and defect location and clinical evaluations were available only from five pairs. Further limitations are the different follow-up intervals and the lack of direct histological evaluation of GAG content in repair tissue. Due to ethical guidelines, histological samples can be taken only if the patient has pain in the operated knee or if there is a new trauma. Since this is very rare, it is very difficult to obtain histological evaluation of GAG content in repair tissue.

With the assumption that histological evaluations of biopsies from repair tissue reveal more fibrocartilage after BMS techniques\textsuperscript{33,38}, and more hyaline-like tissue after MACT\textsuperscript{39-41}, our results may indicate that sodium MRI is able to distinguish not only between native hyaline cartilage and repair tissue, but also between the different qualities of repair tissue after MACT and BMS techniques. As our results suggest, the MACT treatment provides higher GAG content, and therefore, repair tissue of better quality compared to the BMS techniques, and that sodium MRI at 7 T might be beneficial in the non-invasive evaluation of cartilage repair surgery efficacy.

**Author contributions**

The authors declare the following contributions to the preparation of the manuscript: Study conception and design (Welsch,
Mayerhoefer, Szomolanyi, Bogner, Trattnig); collection and assembly of data (Zbýn, Negrin, Domayer); analysis and interpretation of data (Zbýn, Stelzeneder, Juras, Weber, Trattnig); drafting of the manuscript (Zbýn, Stelzeneder, Trattnig); critical revision of the manuscript for important intellectual content (Welsch, Negrin, Juras, Mayerhoefer, Szomolanyi, Bogner, Domayer, Weber); final approval of the article (Zbýn, Stelzeneder, Welsch, Negrin, Juras, Mayerhoefer, Bogner, Domayer, Weber, Trattnig); obtaining of funding (Bogner, Domayer, Trattnig). All authors take responsibility for the integrity of the work.

Conflict of interest
None of the authors have any conflict of interest relating to the submitted manuscript.

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References


