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Tissue-engineered medical products — MRI evaluation of cartilage —

Part 1: Clinical evaluation of regenerative knee articular cartilage using delayed gadolimium-enhanced MRI of cartilage (dGEMRIC) and T2 mapping

Produits médicaux issus de l'ingénierie tissulaire — Évaluation du cartilage par IRM —

Partie 1: Évaluation clinique de la régénération du cartilage articulaire du genou par séquences IRM tardives après injection de gadolinium (dGEMRIC) et cartographie T2

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Foreword

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This document was prepared by Technical Committee ISO/TC 150, *Implants for surgery*, Subcommittee SC 7, *Tissue-engineered medical products*.

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Introduction

Tissue-engineered cartilage has shown desirable results for the repair of cartilage defects, and histologic findings indicate that the repaired tissue has a hyaline-like cartilage structure. Kang H.J. et al., Zheng M.H. et al. and Behrens P. et al. reported that the histologic change after matrix-associated autologous chondrocyte implantation/transplantation (MACI/MACT)[1-3] was a hyaline-like cartilage. The knee articular cartilage can also be repaired or regenerated via other tissue engineering approaches using other seed cells such as mesenchymal stem cells or even by tissue regeneration free of external seed cells[4-6]. MACI and other approaches lead to a maturation of the cartilage matrix over time with the development of an organized collagen architecture. For long-term follow-up of regenerative cartilage, clinical scores and morphological evaluations are commonly used. Furthermore, histological evaluation from arthroscopic biopsies provides a gold standard for morphological and biochemical assessments of regenerative cartilage tissue. However, this process is invasive and unacceptable for patients after cartilage repair surgery. Magnetic resonance (MR) is a noninvasive technique that can be used for the evaluation of a cartilage microstructure. Xu X and other researchers reported that MR-based biochemical imaging techniques, such as delayed gadolinium-enhanced MRI of the cartilage (dGEMRIC) and T2 mapping, show the capability of evaluating the biochemical character of articular cartilage[7-12]. The T2 relaxation time is sensitive to the content of effective hydrogen atoms, and thus to the concentration of collagen, the main component of cartilage extracellular matrix[13]. Besides, the orientation changes in the collagen network of articular cartilage produce the depthwise T2 anisotropy through the magic angle effect[14]. The dGEMRIC technique enables an indirect estimation of the fixed charge density (FCD) of cartilage, which mainly arises from the aggregated proteoglycan biomacromolecules[15]. Since both collagen and proteoglycan components are important for determining the functional characteristics of cartilage, a combination of T2 mapping and dGEMRIC techniques provides a better evaluation of articular regenerative cartilage. Therefore, standardization of T2 mapping and dGEMRIC techniques is needed for the evaluation of regenerative articular cartilage.

This document is intended to guide the clinical biochemical evaluation of regenerative articular cartilage with MR. dGEMRIC and T2 mapping are recommended for the clinical evaluation of regenerative cartilage. These techniques have been used for patients who received tissue-engineered cartilage implantation or transplantation (MACI/MACT). The validation data from different hospitals are provided Annex A.

This document provides general principles for imaging and the measurement method of T2 mapping and dGEMRIC of knee cartilage using 1,5 T or 3,0 T MRI equipment. These techniques are also applicable for other articular cartilage, such as the ankle joint, hip joint, and shoulder joint, but the imaging parameters should be adjusted and modified for better image quality.

Tissue-engineered medical products — MRI evaluation of cartilage —

Part 1: Clinical evaluation of regenerative knee articular cartilage using delayed gadolimium-enhanced MRI of cartilage (dGEMRIC) and T2 mapping

1 Scope

This document provides a principle to determine the parameter settings and operating methods for the evaluation of the composition and structure of articular cartilage by dGEMRIC and T2-mapping MRI in humans with a typical example of the methods; each are distinct MRI technologies that allow for noninvasive observation of soft tissue characteristics.

The methods provided in this document are intended for application in the evaluation of the clinical effects of tissue-engineered cartilage or other cartilage regeneration products used in the knee joint, and are also applicable for the evaluation of regenerative cartilage in other joints, although some modification of parameters is needed.

This document describes a longitudinal evaluation of the water content, the glycosaminoglycan (GAG) concentration, and the concentration and orientation of collagen fibres in regenerative cartilage when using dGEMRIC and T2-mapping techniques in 1,5 T or 3,0 T magnetic resonance imaging equipment.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
pulse sequences
train of programmed radio frequency pulses and gradient pulses

Note 1 to entry: In MRI, it is a time protocol for encoding images to obtain k-space data.

3.2
number of averages
NA
number of repeated acquired identical MR signals from the same programmed pulse sequence

3.3
voxel
three-dimensional cuboid representing the minimum unit comprising a three-dimensional image

3.4
pixel
two-dimensional cuboid representing the minimum unit comprising an image

3.5
field of view
FOV
width and height of an imaged region

Note 1 to entry: It is expressed in cm by cm or mm by mm.

3.6
matrix
array of scalars arranged in frequency encoding direction and phase encoding direction in a two-dimensional MR image

Note 1 to entry: It is typically expressed in number of pixels in frequency encoding direction by number of pixels in phase encoding direction.

Note 2 to entry: In MRI, the scalars in the array are called pixel of the matrix.

3.7
slice thickness
thickness of the imaging plane

Note 1 to entry: It is expressed in cm or mm.

3.8
signal-to-noise ratio
SNR
single number obtained by dividing the image signal by the image noise

3.9
region of interest
ROI
user-defined area on an image in which parameter of interested is calculated

3.10
echo time
TE
time from the centre of the 90-degree excitation RF-pulse to the centre of the echo

Note 1 to entry: It is expressed in ms.

3.11
repetition time
TR
time interval for repetition of the basic unit of magnetic resonance pulse sequences

Note 1 to entry: It is expressed in ms.

3.12
proton density-weighted image
PDWI
magnetic resonance image reflecting the concentration of protons in tissue

3.13
matrix-associated autologous chondrocyte implantation/transplantation
MACI/MACT
procedure involving expansion of autologous chondrocytes and seeding the cells onto a three-dimensional biomaterial scaffold

3.14
scaffold
support or structural component or delivery vehicle, or matrix, consisting of synthetic and/or naturally-derived material(s), for modulating the biological properties or transport of administered and/or endogenous cells and/or binding/transport of bioactive agents

Note 1 to entry: Biological properties include (but are not limited to) adhesion, migration, proliferation, and differentiation.

[SOURCE: ASTM F2312-11:2020, Clause 4]

3.15
gradient recalled echo
GRE
MR sequence that generates gradient echoes as a consequence of echo refocusing

3.16
delayedgadolinium enhanced MRI of the cartilage
dGEMRIC
pre-contrast and post-contrast T1 mapping of cartilage

3.17
longitudinal relaxation time
T1
time taking for the longitudinal magnetization to recover approximately 63 % of its initial value after being flipped into the magnetic transverse plane by a 90° radiofrequency pulse

Note 1 to entry: It is expressed in ms.

3.18
transverse relaxation time
T2
time taking for the magnetic resonance signal to irreversibly decay to 37 % of its initial value after being flipped into the magnetic transverse plane by a 90° radiofrequency pulse

Note 1 to entry: It is expressed in ms.

3.19
T1 mapping
two-dimensional spatial distributions of T1 value of tissue

3.20
T2 mapping
two-dimensional spatial distributions of T2 value of tissue

3.21
R1
longitudinal relaxation rate calculated as 1/T1

4 Principles

Articular cartilage is a type of hyaline cartilage that is characterized by an extracellular matrix that contains a fine network of collagen and proteoglycan[16]. In regenerative articular cartilage, it is important to evaluate whether the implanted tissues regenerate to hyaline or hyaline-like cartilage with time. MRI is a noninvasive technique that can provide an indirect method for assessing the composition and microstructure of articular regenerative cartilage, including content and organization of the collagen network and the proteoglycan, as the main component in the extracellular matrix[12],[17].

Delayed gadolinium enhanced MRI of the cartilage (dGEMRIC) is a technique pertinent to the T1 relaxation-time measurement that uses the negative ionic charge of gadopentetate dimeglumine (Gd-

DTPA^{2-}) to map the fixed charge density of the cartilage GAG. Gd-DTPA^{2-} is repelled by negatively charged GAGs and is therefore negatively related to the local proteoglycan concentration. Consequently, Gd-DTPA^{2-} accumulates in areas of low GAG content, and a cartilage will have a shorter T1 relaxation time in these regions. The ability to measure spatial variations in the cartilage GAG concentration in vitro with dGEMRIC has been validated biochemically and histologically using both bovine and human cartilage. The feasibility of using dGEMRIC in vivo has also been demonstrated, and the interpretation of MR images as representing a GAG distribution is supported by literature evidence[18-21]. The GAG is a component of normal hyaline cartilage that is critical to its mechanical strength. Thus, as a noninvasive method of indirectly monitoring the GAG concentration in cartilage, dGEMRIC is a potentially useful method for assessing regenerative cartilage.

T2 mapping usually involves imaging at several echo times along the T2 decay curve[22] and T2 relaxation time of different tissues can be calculated after data processing. In cartilage, changes in the T2-relaxation times are dependent upon the quantity of water and the integrity of the proteoglycan–collagen matrix. T2 relaxation time mapping provides an indirect assessment of the collagen structure and orientation as it relates to the free water content. The presence of unbound water molecules slows the loss of transverse magnetization following an RF pulse, such that regions of cartilage with more free water have higher T2 relaxation times. In healthy cartilage, the collagen matrix traps and immobilizes water molecules. When this structured matrix breaks down, the extra space is filled with free, unbound water, and leads to elevated T2 relaxation times. The correlation between T2 relaxation time mapping and the collagen content has been validated, both in vitro and in vivo[23],[24]. The T2 value of cartilage is a dipolar interaction due to the slow anisotropic motion of water molecules in the collagen matrix and varies as a function of the collagen arrangement in the static magnetic field[14],[25], the strength of this interaction is orientation-dependent and reaches its minimum at an angle of 54,7 (between the static field and the axis of interacting protons, the so-called “magic angle”. Consequently, T2 changes along cartilage thickness are reported to follow the orientational changes in the collagen fibril network. Using appropriate arrangement of the articular surface with respect to the B0 field the resulting laminated appearance in T2 maps approximately corresponds to the histological collagenous zones: the superficial zone (orientation of collagen fibrils parallel to the articular surface), the transitional zone (random fibril orientation) and the deep or radial zone (fibrils perpendicular to the articular surface and perpendicular to the bone), which reveals the spatial collagen architecture in articular cartilage. This spatial variation is a marker for hyaline-like matrix organization after cartilage repair.

MACI/MACT uses biomaterial scaffolds (natural or synthetic materials) as a carrier and seeds cells of autologous chondrocytes. The repaired tissue can develop an organized collagen network, which is the basis for histological characterization of normal hyaline articular cartilage over time[1-3],[26],[27]. It is possible to longitudinally evaluate the water content, the GAG concentration, and the concentration and orientation of collagen fibres in regenerative cartilage after MACI/MACT by using the dGEMRIC and T2 mapping techniques.

In this document, T2 mapping and dGEMRIC data obtained from subjects who received MACI using different MRI equipment are included Annex A.

5 T2 mapping evaluation in human knee articular cartilage

5.1 Characterization parameters and methods

The 1,5 T or 3,0 T magnetic resonance imaging equipment and multichannel phased-array knee coil are recommended for T2 mapping examination of knee cartilage. It is recommended to use the same field strength equipment for longitudinal evaluation to avoid the influence of static magnetic field B0 on the relaxation time of the tissue. Before MRI examinations, the subject should rest for more than 30 min to avoid mechanical loading by exercise, which can influence the T2 value of knee cartilage. B0 and B1 shimming is highly recommended before scanning the T2-mapping sequence for every patient. Sagittal proton density-weighted images with fat saturation (FS-PDWI) and three-dimensional gradient recalled echo (3D-GRE) pulse sequences are recommended for morphological evaluation of cartilage. 3D-GRE pulse sequences with spoiled gradient (such as SPGR, FLASH, and VIBE) or steady-state free precession (such as DESS) can be chosen in different MR manufactures. The pixel size in plane of the

3D-GRE pulse sequence should be consistent with pixel size in plane of the T2 mapping sequence, which can ensure the accuracy of the image fusion registration.

A regularly repeated phantom test is recommended to ensure the status and stability of the MR system. Phantom-based quality control is required after any change in the MR system hardware and software.

The protocol of T2 mapping consists of a sagittal, multi-echo spin echo pulse sequence for T2 measurement. Table 1 lists the recommended imaging parameters of T2 mapping in 1,5 T and 3,0 T MR equipment, as a reference.

Table 1 — Recommended Magnetic resonance parameters of T2 mapping evaluation

Parameters	T2 mapping	
	1,5 T	3,0 T
FOV (mm x mm)	160 × 160	160 × 160
TR (ms)	range 1 200 to 2 000	range 1 200 to 2 000
TE (ms)	multiple TE (no less than 4 echo times), more echo times corresponds to more accurate T2 calculation, and the maximum echo time should be shorter than 80 ms	multiple TE (no less than 4 echo times), more echo times corresponds to more accurate T2 calculation, and the maximum echo time should be shorter than 80 ms
Parallel acquisition	the acceleration factor should be no larger than 2	the acceleration factor should be no larger than 2
Matrix	no less than 256 × 256	no less than 320 × 320
Pixel size in plane (mm^2)	no larger than 0,6 × 0,6	no larger than 0,5 × 0,5
Number of averages (NA)	1 or 2	1 or 2
Slice thickness (mm)	3 is recommended (ranging 3,0 to 4,0)	3 is recommended (ranging 3,0 to 4,0)
Image plane	sagittal plane	sagittal plane
Number of slices	no more than 30 slices	no more than 30 slices
NOTE The parameters were suggested to be adjusted with different MR equipment and different signal-receiving coil.		

MR examination of PDWI and T1-weighted 3D-GRE pulse sequences should achieve the following standards:

a) the field of view (FOV) should be no larger than 160 mm × 160 mm and no smaller than 140 mm × 140 mm;

b) the pixel size in plane of the PDWI pulse sequence should not be larger than 0,5 mm × 0,5 mm in 3,0 Tesla MRI equipment and should not be larger than 0,6 mm x 0,6 mm in 1,5 Tesla MRI equipment;

c) a 3,0-4,0 mm slice thickness is suggested in the PDWI pulse sequence;

d) for image matching, some parameters, such as FOV, the scanning centre and slice thickness, are suggested to be kept the same for both PDWI and T2 mapping;

e) the voxel size of the 3D-GRE pulse sequence should be isotropic and not larger than 0,5 mm × 0,5 mm × 0,5 mm in 3,0 Tesla MRI equipment and should not be larger than 0,6 mm × 0,6 mm × 0,6 mm in 1,5 Tesla MRI equipment;

f) the fat-saturation technique is suggested in PDWI and 3D-GRE pulse sequences, such as water-excitation or fat water separation methods;

g) imaging with high resolution can require multiple signal averages in 1,5 Tesla MR equipment for a higher signal-to-noise ratio (SNR);

h) if images are acquired with fat suppression, lowering the imaging bandwidth improves the overall SNR.

5.2 T2 value measurement process

5.2.1 Post-processing of imaging

Post-processing of the multiple images generated by the T2 mapping sequences can be performed online on the scanner or offline using algorithms written in separate programs, such as MATLAB (the MathworksInc, Natick, MA). Automated processing on the scanner typically generates a pixel-by-pixel map of T2 relaxation times, and the T2 maps can be overlain on anatomical images through image registration. Generally, sagittal PDW images and 3D-GRE images are recommended for morphological evaluation of regenerative cartilage and native cartilage. PDW images are sensitive to the signal abnormality of regenerative tissue, and 3D-GRE pulse sequence is used to obtain anatomical images for its high resolution. T2 map images can be registered to 3D GRE images for verification of regenerative cartilage and native cartilage (see Figure 1).

5.2.2 Measurement method

T2 relaxation time is obtained by pixel-wise mono-exponential fitting of signal decay at different echo times, and discarding the first echo for curve fitting is recommended in post-processing to minimize the error in T2[28]. If the regenerative cartilage showed longer T2 component not covered by the entire ETL, bi-exponential curves including the offset as an additional parameter should be applied and the corresponding model can be manually selected in the MATLAB software for imaging processing.

The SE pulse sequence signal intensity (S) shall be calculated by Formula (1).

$$S = M_0 \times (1 - exp(-TR / T1)) \times exp(-TE / T2) \quad (1)$$

where

S is the SE pulse sequence signal intensity;

M_0 is equilibrium longitudinal magnetization;

TR is the repetition time;

$T1$ is the longitudinal relaxation time;

TE is the echo time;

$T2$ is the transverse relaxation time.

When TR>>T1, (1-exp(-TR/T1)) approaches 1. When TR is not much longer than T1(mostly in multi echo spin echo T2 mapping sequence), TR is fixed, and the T1 value of the tissue is also relatively fixed according to TR, thus, 1-exp(-TR/T1) is relatively constant with multiple TEs. The M_0×(1-exp(-TR/T1)) can be calculated as constant S_0. Therefore, the above formula can be simplified as Formula (2):

$$S = S_0 \times exp(-TE / T2) \quad (2)$$

where

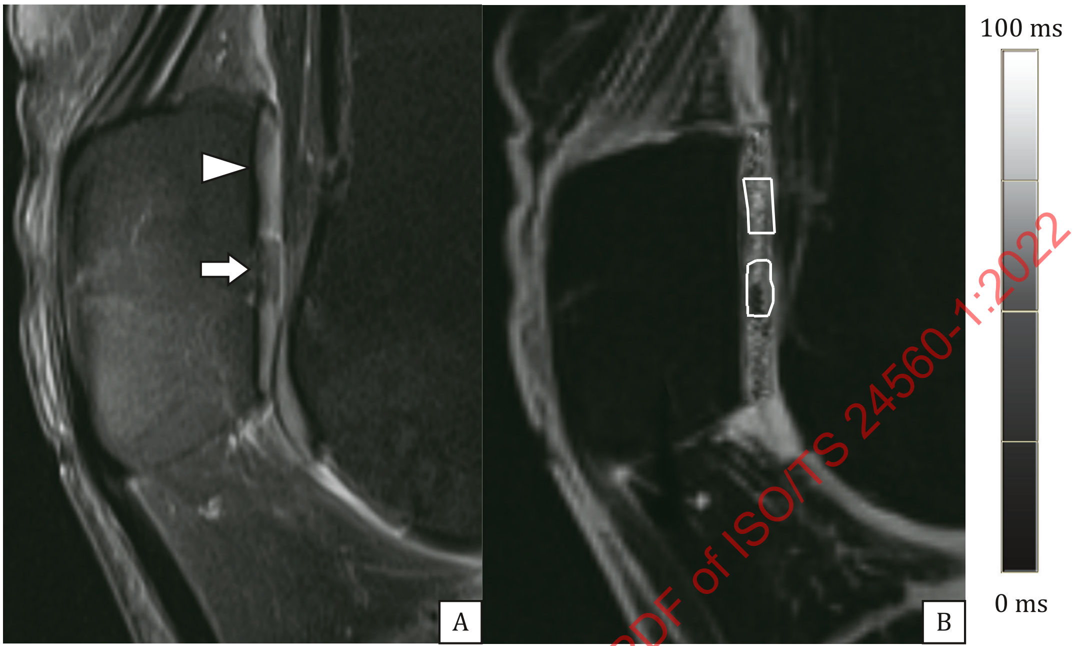
S is the SE sequence signal intensity;

S_0 is the steady longitudinal magnetization during TR recovery after the RF pulse;

$T2$ is the transverse relaxation time;

TE is the echo time.

And the T2 value is calculated by curve fitting.



Key

A	high-resolution FS-PDW image of cartilage in a patient at 6 months after MACI of the patella
B	T2 map matched anatomical image (3D-GRE) of cartilage in a patient at 6 months after MACI of the patella
white arrow	the location of regenerative cartilage
white triangle	the location of control cartilage

NOTE 1 ROIs are placed in T2 map matched anatomical image to locate regenerative cartilage and control cartilage and to measure the T2 values.

NOTE 2 The scale bar of the T2 maps shows the T2 values ranging from 0 ms to 100 ms. The white represents a high T2 value, and the black represents a low T2 value. Besides, the coloured scale bar is also recommended.

Figure 1 — A typical T2-mapping MR image of the knee joint in a patient to demonstrate the measurement of T2 values

After pixel-by-pixel map of T2 relaxation times with either a colour or grayscale map are generated, T2 values of regenerative cartilage and healthy cartilage are measured on T2 maps. The regions of interest (ROIs) are placed in T2 map matched anatomical image to locate regenerative cartilage and control cartilage and to measure the T2 values (see Figure 1).

5.2.3 ROIs of regenerative cartilage

ROIs of regenerative cartilage are drawn manually by an experienced senior musculoskeletal radiologist. The location and extent of regenerative cartilage are identified by at least two radiologists to ensure the accuracy of the ROI placement; in addition, it is recommended that the same radiologists perform a longitudinal evaluation to ensure consistency in the placement of ROIs. For imaging analysis, the ROI of regenerative cartilage should cover the full thickness of the cartilage. In the slice of implanted plugs, the ROI is placed between the edges of each plug (see Figure 1).

5.2.4 ROIs of normal control cartilage

To compare the T2 values between regenerative cartilage and healthy cartilage, a region of morphologically normal-appearing cartilage within the same anatomical region should be selected as a reference (control) cartilage, which is defined as a normal signal on the PDW images if the cartilage thickness is preserved, the surface is intact, and no intrachondral signal alterations are visible[29].

5.3 T2 value evaluation

5.3.1 Purpose of evaluation

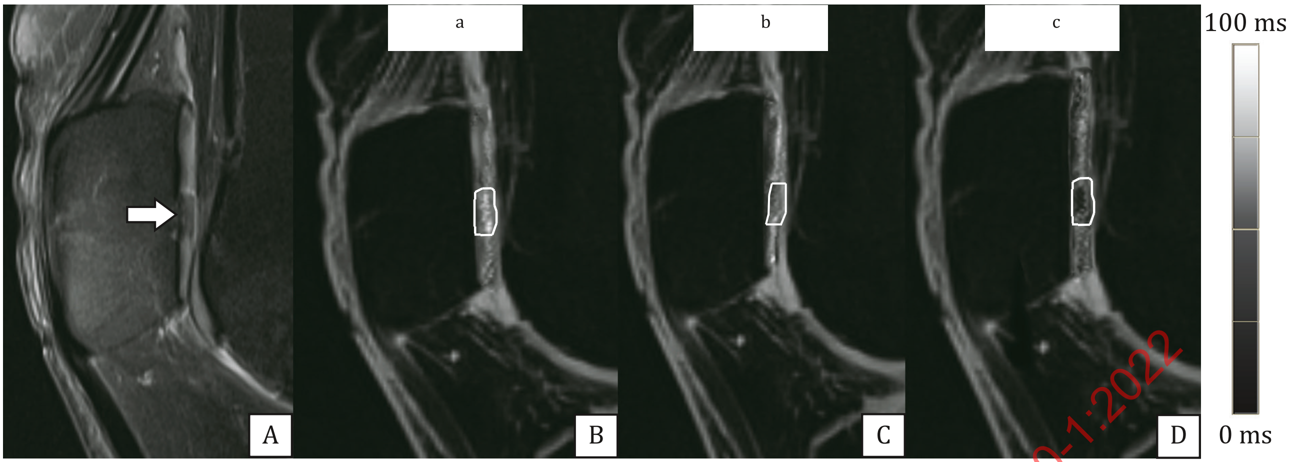
The T2 relaxation time is sensitive to the content of effective hydrogen atoms (mostly in water), and thus to the concentration and orientation of collagen, the main component of cartilage extracellular matrix.

To longitudinally evaluate the water content and collagen fibre orientation of cartilage with T2 mapping, a 1-year follow-up MR examination of T2 mapping is suggested, and the time points for follow up at 3, 6, and 12 months after MACI are recommended. T2 values of both regenerative cartilage and its neighbour normal cartilage (control) should be measured, including 3, 6, and 12 months after MACI. To avoid the influence of static magnetic field B0 on the relaxation time of the tissue, this document suggests the longitudinal evaluation of the articular cartilage in the same equipment or different equipment with the same field strength.

5.3.2 In vivo evaluation of regenerative cartilage with T2 value

For longitudinal evaluation the biochemical change of regenerative knee articular cartilage, the variation tendency of the T2 values of regenerative cartilage should be assessed, and statistical analysis should be performed 3, 6 and 12 months after MACI when the sample size is sufficient (multiple patients) to prove the clinical efficacy of the tissue-engineered product. In addition, to compare the biochemical microstructure of the regenerative cartilage and the native cartilage, a horizontal comparison is recommended between the T2 values of the regenerative cartilage and those of the control cartilage at 3, 6, and 12 months after MACI, and the statistical analysis should be performed when the sample size (multiple patients) is sufficient for a clinical effect evaluation of cartilage regenerative products or for a clinical trial.

When the regenerative tissues undergo a gradually hyaline-like repair, the T2 values of the repair tissue should show a downward trend between 3, 6 and 12 months after MACI. The T2 values of the repaired tissue should be significantly higher than those of the control cartilage 6 months after MACI, which reflect greater hydration and an unorganized collagen network in the regenerative cartilage. If the T2 values show no significant difference between the plugs and the control cartilage 1 year after MACI, it can indicate the maturation of the collagen network and water content of the repaired tissue. This result can indirectly demonstrate that repaired tissues can develop a hyaline-like structure (see Figure 2).



Key

A	the location of regenerative cartilage (white arrow)
B, C and D	T2 map matched anatomical image (3D-GRE) of cartilage in a patient at 3, 6 and 12 months after MACI
white arrow	the location of regenerative cartilage
a	3 months
b	6 months
c	12 months

NOTE The scale bar of the T2 maps shows the T2 values ranging from 0 ms to 100 ms. The white represents a high T2 value, and the black represents a low T2 value. Besides, the coloured scale bar is also recommended.

Figure 2 — Example of in vivo evaluation of regenerative cartilage with T2 values

6 dGEMRIC evaluation in human knee articular cartilage

6.1 Characterization parameters and methods

The dGEMRIC technique is based on the measurement of the T1 relaxation time enhanced by delayed administration of Gd-DTPA^{2-} and is currently the most widely used method for analysing GAG depletion in articular cartilage, which have provided valuable results in vitro and in vivo[20],[30-32]. It contains both pre-contrast T1 mapping and post-contrast T1 mapping. There are many different methods for creating T1 relaxation time maps based on progressive inversion or saturation of longitudinal magnetization[33-37]. The classical method of T1 mapping uses an inversion-recovery prepared fast spin-echo (IR-FSE) T1-weighted pulse sequence with multiple TI (inversion times) to measure the T1 relaxation time[38]; however, the acquisition times required for T1 maps are usually long and are often limited to a small number of slice locations. Recently, the 3D GRE T1-weighted pulse sequence is gradually used in T1 mapping for greater image coverage and a faster acquisition time[39-41]. This document recommends a 3D GRE T1-weighted pulse sequence with variable flip angles in the dGEMRIC technique. This method allowed the acquisition of a slab covering a whole compartment of the knee joint with a relatively high resolution within clinically acceptable scan times[42]. As shown in a phantom study, central positioning of the 3D GRE slab is critical for achieving the best results of T1 mapping for eliminating partial volume effects and increasing the SNR. When this positioning is performed, a good correlation between the variable flip angle technique and the standard inversion recovery technique for T1 mapping has been established in phantoms and in vivo[41],[42].

The 1,5 T or 3,0 T magnetic resonance equipment and multichannel phased-array knee coil are recommended for T1-mapping examination of the knee joint. B0 and B1 shimming are highly recommended before scanning the T1-mapping sequence for every patient. Sagittal PDW images and 3D-GRE images are recommended for morphological evaluation of regenerative cartilage and native cartilage. PDW images are sensitive to the signal abnormality of regenerative tissue, and 3D-GRE pulse sequence is used to obtain anatomical images for its high resolution. T1 mapping (3D GRE T1-weighted pulse sequence with variable flip angle) is performed both before and after slow manual intravenous injection of Gd-DTPA^{2-} (0,2 mM/kg body weight, Magnevist, Schering, Germany), and the Gd-DOTA^{-} can be an alternative when Gd-DTPA^{2-} cannot be used[43,44]. To optimize the penetration of Gd-DTPA^{2-} into knee cartilage, patients are asked to flex and extend the knee joint (walk or other motion) for approximately 10-15 min. Post-contrast T1 mapping is then assessed 90-120 min after the injection until complete diffusion of the contrast agent into the cartilage.

A regularly repeated phantom test is recommended to ensure the status and stability of the MR system. Phantom-based quality control is required after any change of the MR system hardware and software.

The protocol of T1 mapping consists of a sagittal, multi-flip angle 3D spoiled GRE T1-weighted pulse sequence. Table 2 lists the imaging parameters of T1 mapping in 1,5 T and 3,0 T MR equipments, just as a reference.

Table 2 — Recommended Magnetic resonance parameters of T1 mapping evaluation

Parameters	T1 mapping	
	1,5 T	3,0 T
FOV (mm x mm)	160 × 160	160 × 160
TR (ms)	ranging 6 to 50 (depend on MR equipment)	ranging 7 to 50 (depend on MR equipment)
TE (ms)	minimum echo time	minimum echo time
Flip angles	at least 2 flip angles, and 3 or 4 flip angles are recommended	at least 2 flip angles, and 3 or 4 flip angles are recommended
Parallel acquisition	the acceleration factor should be no larger than 2	the acceleration factor should be no larger than 2
Matrix	no less than 256 × 256	no less than 320 × 320
Pixel size in plane (mm^2)	no larger than 0,6 × 0,6	no larger than 0,5 × 0,5
Number of averages	1 or 2	1 or 2
Slice thickness (mm)	3 is recommended (ranging 3,0 to 4,0)	3 is recommended (ranging 3,0 to 4,0)
Image plane	sagittal plane	sagittal plane
Number of slices	no more than 30 slices	no more than 30 slices
NOTE 1 The parameters are suggested to be adjusted with different MR equipments and different signal-receiving coils.		
NOTE 2 Two flip angles can be used in T1 mapping with the benefit of saving scan time but show the following limitation: 1) it is possible that two flip angles are not enough to get accurate T1 value for a large range of T1 relaxation time; 2) it is susceptible to patient motions, and that is a significant downside for longitudinal analysis.		

MR examination of PDWI should achieve the following standards:

a) the FOV should be no larger than 160 × 160 mm and no smaller than 140 mm × 140 mm;

b) the pixel size in plane of the PDWI pulse sequence should not be larger than 0,5 mm × 0,5 mm in 3,0 Tesla MRI equipment, and should not be larger than 0,6 mm x 0,6 mm in the 1,5 Tesla MRI equipment;

c) a slice thickness from 3,0 mm to 4,0 mm is suggested for the PDWI pulse sequence;

d) for image matching, some of the parameters, such as FOV, the scanning centre and slice thickness, are suggested to be kept the same for both PDWI and T1 mapping;

e) a fat-saturation technique is suggested for PDWI, such as water-excitation or fat water separation methods;

f) imaging with high resolution can require multiple signal averages in 1,5 Tesla MR equipment for a higher signal-to-noise ratio (SNR);

g) if imaging is done with fat suppression, lowering the imaging bandwidth improves the overall SNR.

6.2 T1 value measurement process

6.2.1 Post-processing of imaging

Post-processing of the multiple images generated by the T1 mapping sequences can be performed online on the scanner or offline using algorithms written in separate programs, such as MATLAB (the Mathworks Inc, Natick, MA), and the formulas of calculating T1 values are indicated in 6.2.2. Automated processing on the scanner typically generates a pixel-by-pixel map of T1 relaxation times, and the T1 maps can be overlain on anatomical images through image registration. Generally, sagittal PDW images and 3D-GRE images are recommended for morphological evaluation of regenerative cartilage and native cartilage. PDW images are sensitive to the signal abnormality of regenerative tissue, and 3D-GRE pulse sequence is used to obtain anatomical images for its high resolution. T1 map images can be registered to 3D GRE images for verification of regenerative cartilage and native cartilage.

6.2.2 Measurement method

3D-GRE pulse sequences with spoiled gradient (such as SPGR, FLASH, and VIBE) can be used as T1 mapping sequences. The spoiled GRE pulse sequence with multiple flip angles is used to calculate the T1 relaxation times. The spoiled GRE signal intensity shall be calculated using Formula (3).

$$S_{spoiled\ GRE} = \frac{M_0\left(1-E_1 exp\left(-TE/T2^*\right)\right)\sin\alpha}{1-E_1\cos\alpha} \qquad (3)$$

where

M_0 is equilibrium longitudinal magnetization;

E_1 is obtained by the formula exp(-TR/T1);

TR is the repetition time;

$T1$ is the longitudinal relaxation time;

TE is the echo time;

$T2$ is the transverse relaxation time;

α is the flip angle.

Here, E_1 = exp(-TR/T1), and minimum TE which is much smaller than T2* is used in the spoiled GRE pulse sequence, Formula (3) can be represented in the linear form, Y = mX+b, shown as Formula (4).

$$\frac{S_{spoiled\ GRE}}{\sin\alpha} = E_1 \frac{S_{spoiled\ GRE}}{\tan\alpha} + M_0\left(1-E_1\right) \qquad (4)$$

From which the slope *m is* E_1, and the Y-intercept *b is* $M_0(1\text{-}E_1)$, can be estimated by regression, allowing T1 and M_0 to be extracted:

$$T1 = -\mathrm{TR}/\ln m \qquad (5)$$

$$M_0 = b/(1-m) \tag{6}$$

So, the T1 mapping can be calculated by two or more flip angles. Using two flip angles, the system calculates the slope m and Y-intercept by those two points. The linear fitting with the least-square algorithm is done when the available number of flip angles is more than two[45,46].

After a series of pixel-by-pixel map of T1 relaxation times are generated, T1 values of regenerative cartilage and healthy cartilage are measured on T1 maps. Regions of interest (ROIs) are placed in the native and regenerative cartilage to measure the T1 values.

6.2.3 ROIs of regenerative cartilage

The ROIs of regenerative cartilage are drawn manually by an experienced senior musculoskeletal radiologist. The location and extent of regenerative cartilage are identified by at least two radiologists to ensure accuracy in the placement of the ROI; in addition, it is recommended that the same radiologists perform a longitudinal evaluation to ensure consistency in the placement of ROIs. For imaging analysis, the ROI of regenerative cartilage should cover the full thickness of cartilage. In the slice of implanted plugs, the ROI is placed between the edges of each plug.

6.2.4 ROIs of normal control cartilage

To compare the T1 values between regenerative cartilage and healthy cartilage, a region of morphologically normal-appearing cartilage within the same anatomical region should be selected as a reference (control) cartilage, which is defined as a normal signal with a preserved thickness and intact surface on FS-PDWI images.

6.3 ΔR1 value calculation

T1 values are used to calculate the difference between R1pre and R1post (ΔR1) for regenerative cartilage and control cartilage.

$$\Delta R1 = R1post - R1pre \tag{7}$$

where

- *R1* is 1 divided by the T1 (typically expressed 1/s);
- *R1pre* is 1 divided by the pre-contrast T1;
- *R1post* is 1 divided by the post-contrast T1.

In this document, ΔR1 values are used to evaluate the GAG content of cartilage. An agreement between the GAG content and ΔR1 in the knee is reported and is demonstrated by liquid chromatography of repaired tissue biopsies after ACI[38],[47].

6.4 ΔR1 value evaluation

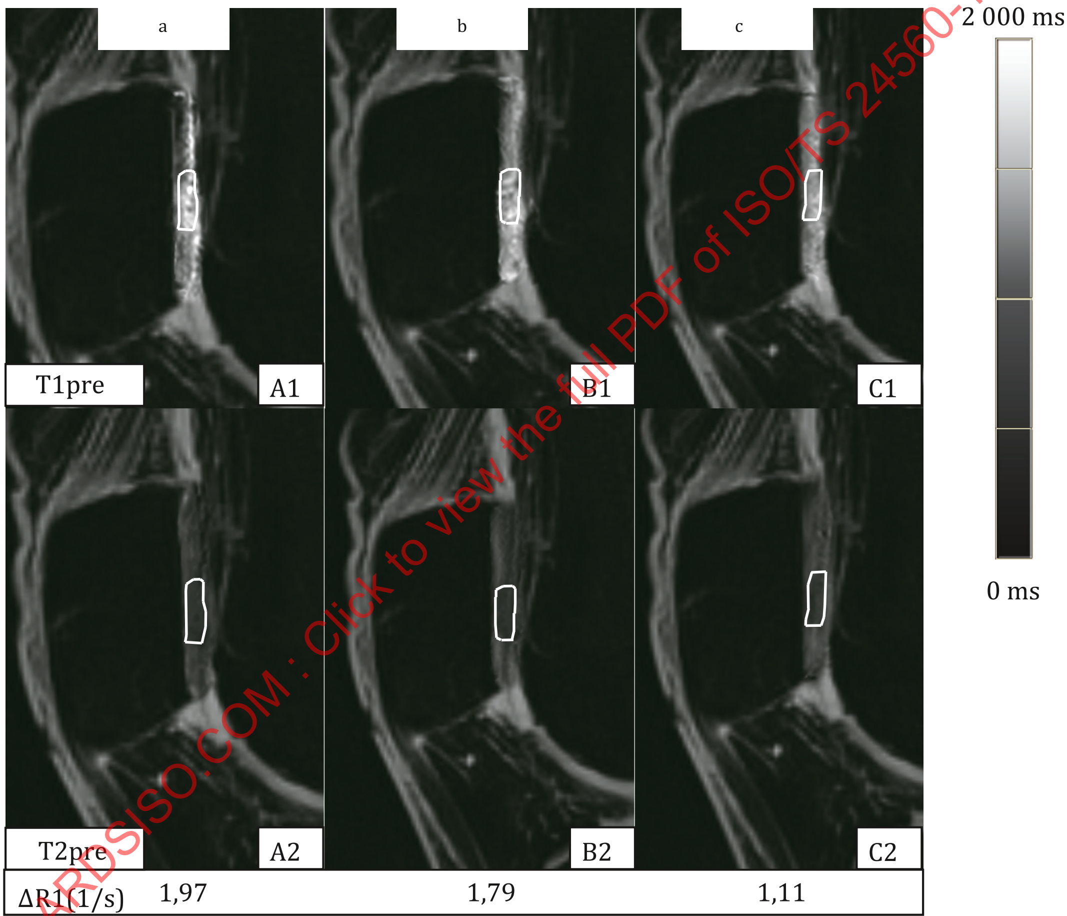
6.4.1 Purpose of evaluation

The ΔR1 value is sensitive to the GAG concentration in cartilage. There is a significant negative correlation between the relative ΔR1 and the relative GAG concentration.

To longitudinally evaluate the GAG concentration in cartilage, a 1-year follow-up MR examination of dGEMRIC is suggested, and 3, 6, and 12 months after MACI are recommended. ΔR1 values of regenerative cartilage and normal control cartilage should both be measured, including 3, 6, and 12 months after MACI. Because the T1 values of tissues are different between 1.5T and 3.0T, this document suggests the longitudinal evaluation of the articular cartilage in the same equipment or in different equipments with the same field strength.

6.4.2 In vivo evaluation of regenerative cartilage with ΔR1 values

For longitudinal evaluation of the biochemical change of regenerative knee articular cartilage, the variation tendency of the ΔR1 values of regenerative cartilage should be assessed, and statistical analyses should be performed 3, 6 and 12 months after MACI, when the sample size is sufficient (multiple patients), to prove the clinical efficacy of the tissue-engineered product. In addition, to compare the biochemical microstructure of the regenerative cartilage and native cartilage, a horizontal comparison is recommended for a comparison of the ΔR1 values between regenerative cartilage and control cartilage at 3, 6, and 12 months after MACI, and a statistical analysis should be performed when the sample size (multiple patients) is sufficient.



Key

a 3 months

b 6 months

c 12 months

NOTE 1 Pre- and post-contrast T1 map after 3 months (A1 and 2), 6 months (B1 and 2) and 12 months (C1 and 2) matched anatomical image of regenerative cartilage at different periods after MACI. ROIs mark the area of regenerative cartilage and T1 values of each ROI are measured. The scale bar of T1 maps shows the T1 values ranging from 0 ms to 2 000 ms. The white represents high T1 value, and the black represents low T1 value. Besides, the coloured scale bar is also recommended.

NOTE 2 ΔR1 value are calculated as 1/T1post-1/T1pre, and the downward trend of ΔR1 values after MACI can be observed.

Figure 3 — Example of in vivo evaluation of regenerative cartilage with dGEMRIC

When the regenerative tissue shows gradually hyaline-like repair, the ΔR1 values of regenerative tissue should show a downward trend 3, 6 and 12 months after MACI. ΔR1 values of regenerative tissue should be significantly higher than those of control cartilage 6 months after MACI, which reflects a lower GAG content in regenerative cartilage tissue. However, if the ΔR1 values show no significant difference between the plugs and control cartilage 1 year after MACI, the imaging analysis can indicate a normal GAG content in regenerative cartilage. This result can indirectly demonstrate that regenerative tissues can develop a hyaline-like structure (see Figure 3).

7 Acceptable standard for MR evaluation

7.1 Requirements for MR equipment

In this document, in vivo MR-based biochemical evaluation of articular regenerative cartilage is suggested for application in high field magnetic resonance equipment, such as 1,5 Tesla and 3,0 Tesla MRI equipments, with a multichannel phased-array knee coil.

7.2 Requirements for MR parameters

This document just lists the range of MR parameters of T2 mapping and dGEMRIC. The parameters should be adjusted with a different MR equipment and a different signal-receiving coil. Nevertheless, the standards for T2 mapping and dGEMRIC should meet the criteria in 5.1 and 6.1.

7.3 Requirements for the MR longitudinally evaluation

The regenerative cartilage tissue can develop an organized collagen network, which is the basis for histological characterization of normal hyaline articular cartilage over time. Some articles report that the biomechanical characteristics of regenerative cartilage tissue are similar to those of normal articular cartilage at 1 year after surgery. In this document, a follow up at no less than 1 year after cartilage implantation is suggested, and MR longitudinal evaluations at 3, 6, and 12 months after surgery are proposed.

7.4 Exclusion criteria

Subjects with the following conditions should be excluded from the use of MR evaluations:

a) MR contraindications:

1) cardiac pacemakers, nerve stimulators, insulin pumps, artificial metal heart valves;

2) aneurysm clip (except for non-paramagnetic materials such as titanium alloy);

3) metal foreign body in the eye, metal prosthesis implanted in the inner ear, metal prosthesis, metal joint, and ferromagnetic foreign body in the body;

4) claustrophobia;

5) pregnant women.

b) Contraindications of the Gd contrast agent:

1) chronic, severe kidney disease (glomerular filtration rate, GFR <30 ml/min/1,73 m^2);

2) acute kidney injury;

3) history of severe hypersensitivity reactions to the Gd contrast agent.

8 Limitation

The application of the method provided in this document can have some limitations as follows:

a) this MR evaluation technique of cartilage is mainly applicable to knee cartilage; the resolution (approximately 0,5 mm × 0,5 mm) restricts its utility in some joints with very thin cartilage (less than 0,5 mm × 0,5 mm);

b) the Gd contrast agent is used in the dGEMRIC technique, which can restrict the application of this technique due to these reasons:

   1) nephrogenic systemic fibrosis (NSF), characterized by thickening and hardening of the skin with hyperpigmentation, is a rare but potentially fatal disorder that can occur in patients with severe renal impairment who receive gadolinium-based contrast agent (GBCA)[48],[49];

   2) the deposition of gadolinium in the brain (dentate nucleus and the globus pallidus) is associated with the linear GBCA (Gd-DTPA^{2-}), but not with macrocyclic GBCAs, and the Gd-DOTA^{-} can be an alternative when Gd-DTPA^{2-} cannot be used[50],[51];

   3) dGEMRIC takes relatively long time (post-contrast T1 mapping should be assessed 90-120 min after the injection);

   4) the Gd contrast agent is costly.

In those cases, T1ρ can be used as selective method for evaluating GAG when the sequence is available[52],[53]. Annex B provides a brief introduction of T1ρ technology.

c) Although multicentre validation of the proposed dGEMRIC and T2-mapping methods have been performed in China, as well as can be found in many papers, global multicentre validation needs to be implemented in the future.

Annex A
(informative)

Example of measurement results

A.1 Measurement subjects

In this data acquisition, 13 regenerative cartilage tissues (from 9 patients) underwent T2 mapping and dGEMRIC MR examination at Chinese People's Liberation Army (PLA) General Hospital; an allograft extracellular matrix-derived cartilage scaffold was used for implantation. Table A.1 presents the gender, age, body mass index (BMI), location and size of the cartilage defects of the measured subjects at the time of imaging. In addition, 7 regenerative cartilage tissues (from 7 patients) received only T2-mapping MR examination at the Guangdong Provincial People's Hospital, which are patients who had Type I collagen cartilage matrix (CaReS-1S) without cell implantation. The data are presented in Table A.2. Four regenerative cartilage tissues (from 4 patients) only received dGEMRIC MR examination at the Peking University Shenzhen Hospital, which are patients who had Type I collagen cartilage matrix (CaReS-1S) without cell implantation. The data are presented in Table A.3.

Table A.1 — Gender, age, BMI, location and size of cartilage defects of 9 patients at Chinese PLA General Hospital

Case #	**Gender**	**Age** (year)	**BMI** (kg/m^2)	**Defect size** (cm^2)	**Location**
1	M	40	26,7	1,5	Left medial femoral condyle
				1,6	Right medial femoral condyle
				0,8	Right patella
2	F	50	23,4	2,3	Right patella
				1	Right femoral trochlea
3	F	50	23,8	2	Right patella
				1	Right femoral trochlea
				5	Left patella
4	F	47	22	2,5	Left femoral trochlea
				1,9	Right femoral trochlea
5	F	53	24,2	0,8	Left patella
				1,2	Right patella
6	M	28	29,9	2	Right medial femoral condyle
7	M	57	28,2	1,5	Left patella
8	M	48	26,9	3	Right medial femoral condyle
9	M	49	22,7	0,6	Left femoral trochlea
Mean		46,9	25,3	1,09	
SD		8,4	2,7	0,27	

Table A.2 — Gender, age, BMI, location and size of cartilage defects of 7 patients at the Guangdong Provincial People's Hospital

Case #	Gender	Age (year)	BMI (kg/m^2)	Defect size (cm^2)	Location
1	M	34	26,5	1,5	Left lateral femoral condyle
2	M	50	26,3	1,2	Left femoral trochlea
3	M	22	18,6	1	Right medial femoral condyle
4	M	43	26,1	1,1	Right femoral trochlea
5	F	48	26,3	1,6	Left medial femoral condyle
6	M	41	29,9	2	Right lateral femoral condyle
7	M	35	27,2	1,65	Right medial femoral condyle
Mean		39	25,8	1,4	
SD		9,6	3,5	0,4	

Table A.3 — Gender, age, BMI, location and size of cartilage defects of 4 patients at the Peking University Shenzhen Hospital

Case #	Gender	Age (year)	BMI (kg/m^2)	Defect size (cm^2)	Location
1	M	34	24,9	0,6	Left lateral femoral condyle
2	F	41	26,1	2,0	Right lateral femoral condyle
3	M	29	29,3	0,8	Left lateral femoral condyle
4	M	29	23,1	0,5	Left lateral femoral condyle
Mean		33,25	25,85	0,97	
SD		5,68	2,61	0,69	

Inclusion criteria for patient enrolment are as follows:

a) Grade III to IV lesions, according to the International Cartilage Repair Society scale (ICRS)[54].

b) Cartilage lesions of the femoral condyles, femoral trochlea, tibial plates, or patella in patients with ages of 18 to 60 years.

Exclusion criteria are as follows:

a) Infectious, neoplastic, metabolic and inflammatory changes.

b) Contraindication for MR examination or hypersensitiveness for MR contrast agents.

A.2 T2 mapping evaluation

A.2.1 SKYRA 3,0T (Chinese PLA General Hospital)

The 9 patients at Chinese PLA General Hospital underwent T2-mapping MR imaging 4 times at 1, 3, 6 and 12 months after MACI using a 3,0 Tesla MRI system (MAGNETOM SKYRA, Siemens AG, Erlangen, Germany) that had a gradient strength of 45 mT/m and a 15-channel phased-array knee coil.

Before MRI examinations, the patients were asked to rest for more than 30 min, to avoid the influence of activity. The sagittal 3D-VIBE sequence and sagittal FS-PDWI pulse sequences were acquired for precise localization of the regenerative cartilage. The T2-mapping sequence was performed after axial PDWI and sagittal PDWI. The protocol of T2 mapping consisted of a sagittal, multi-echo T2-weighted sequence. Table A.4 summarizes the imaging parameters used in SKYRA 3,0T.

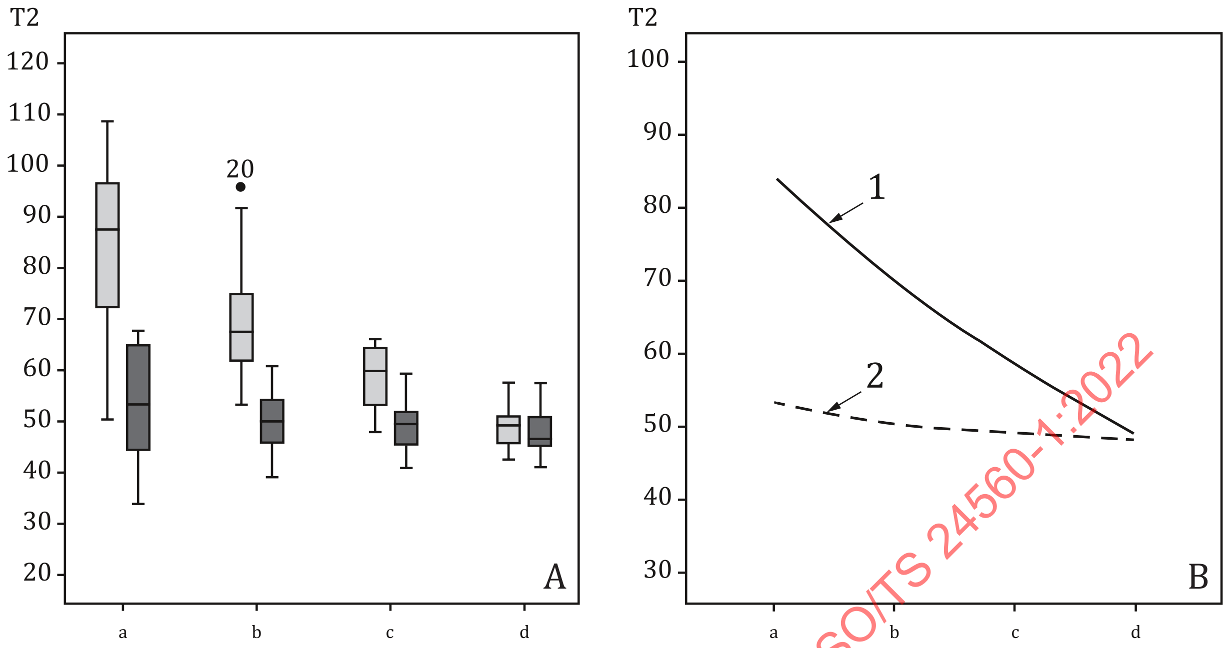
The mean T2 values of the repaired tissues and control cartilage at 1, 3, 6 and 12 months after MACI were measured. The measurement results showed that the T2 values of the plugs showed a downward trend and significant differences 1, 3, 6 and 12 months after MACI (P<0,01). Furthermore, the differences between the T2 values of the regenerative cartilage and those of the control cartilage were smaller with a longer follow-up interval. These results are summarized in Table A.5 and Figure A.1.

Table A.4 — T2 mapping parameters in SKYRA 3,0T

	T2 mapping	3D-VIBE	FS-PDWI
FOV (mm × mm)	160 × 160	160 × 160	160 × 160
TR (ms)	1 921	11,6	3 000
TE (ms)	13,8 27,6 41,4 55,2 69,0	5,4	31
Matrix	384 × 384	384 × 384	384 × 384
Voxel size (mm^3)	0,4 × 0,4 × 3,0	0,4 × 0,4 × 0,4	0,4 × 0,4 × 3,0
Number of averages	1	1	1
Slice thickness (mm)	3,0	0,4	3,0
Image plane	Sagittal plane	Sagittal plane	Sagittal plane
Number of slices	24	160	24

Table A.5 — Mean T2 Values (msec) acquired in SKYRA 3.0T

	1 month	3 months	6 months	12 months
Repair tissues	83,97 ± 16,15	69,78 ± 11,44	58,45 ± 6,63	48,99 ± 3,99
Control cartilage	53,04 ± 11,01	50,20 ± 5,89	48,97 ± 5,77	48,11 ± 4,49
P value	< 0,01	< 0,01	< 0,01	0,51
NOTE Data are presented in mean values ± SD. *P* values refer to the comparison of regenerative cartilage and control cartilage using a t-test.				



Key

T2 T2(ms)

repair tissue (light grey box)

control cartilage (dark grey box)

1 repair tissue

2 control cartilage

a 1 month

b 3 months

c 6 months

d 12 months

Figure A.1 — T2 mapping data acquired in SKYRA 3,0T shown as box plots (A) and line pictures (B)

A.2.2 ESPREE 1,5T (Guangdong Provincial People's Hospital)

The 7 patients at Guangdong Provincial People's Hospital underwent T2 mapping MR imaging 2 times 6 and 12 months after MACI using a 1,5 Tesla MRI system (Siemens Espree) that had a gradient strength of 45 mT/m and a 15-channel phased-array knee coil.

Before the MRI examinations, all subjects were asked to rest for more than 30 min to avoid the influence of activity. The sagittal 3D-VIBE sequence and sagittal FS-PDWI pulse sequences were acquired for precise localization of the regenerative cartilage. The T2-mapping sequence was performed after axial PDWI and sagittal PDWI. The protocol of T2 mapping consisted of a sagittal, multi echo T2-weighted sequence. Table A.6 summarizes the imaging parameters used in ESPREE 1,5T.

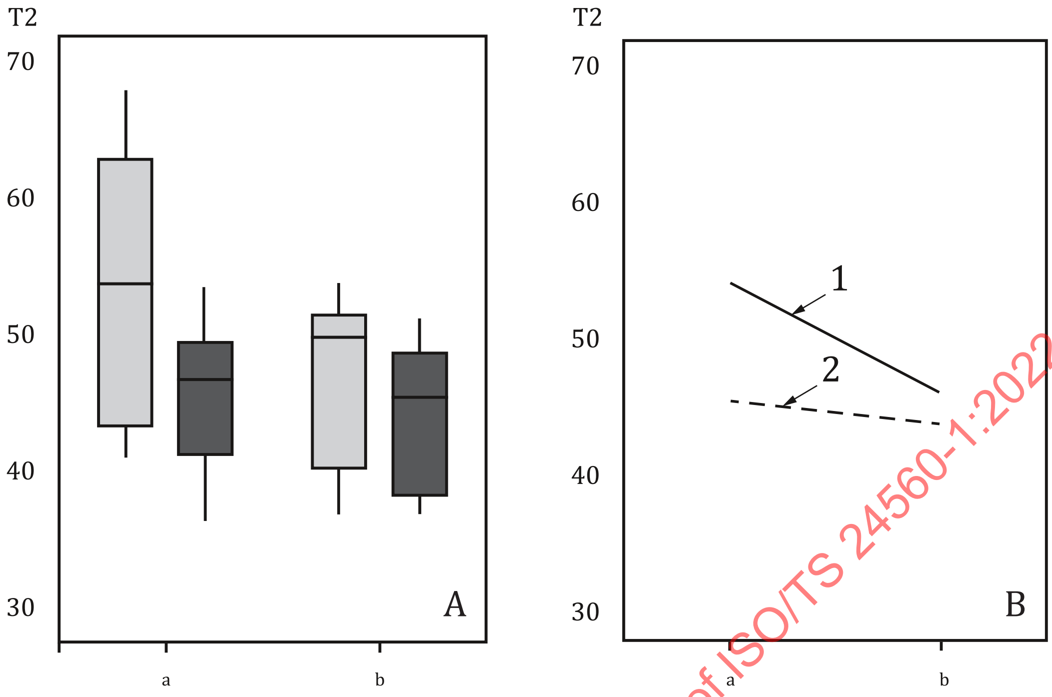
The mean T2 values of the repaired tissues and the control cartilage 6 and 12 months after MACI were measured. The measurement results showed that the mean T2 values of the plugs were significantly higher than those of the control cartilage at 6 and 12 months ($P<0,05$). Furthermore, the differences between the T2 values of the regenerative cartilage and those of the control cartilage were smaller at 12 months after MACI. These results are summarized in Table A.7 and Figure A.2.

Table A.6 — T2 mapping parameters in ESPREE 1,5T

	T2 mapping	3D-VIBE	FS-PDWI
FOV (mm × mm)	160 × 160	160 × 160	160 × 160
TR (ms)	1 460	35	3 320
TE (ms)	14,3 28,6 42,9 57,2 71,5	11	88
Matrix	256 × 256	256 × 256	256 × 256
Voxel size (mm^3)	0,6 × 0,6 × 3,0	0,6 × 0,6 × 0,6	0,6 × 0,6 × 3,0
Number of averages	1	2	2
Slice thickness (mm)	3,0	0,6	3,0
Image plane	Sagittal	Sagittal	Sagittal
Number of slices	24	160	24

Table A.7 — Mean T2 Values (msec) acquired in ESPREE 1,5T

	6 months	12 months
Repair tissues	53,91 ± 9,91	45,70 ± 6,63
Control cartilage	45,02 ± 5,61	43,36 ± 5,95
P value	< 0,01	< 0,05
NOTE Data are presented in mean values ± SD. *P* values refer to the comparison of regenerative cartilage tissues and the corresponding control cartilage using t-test.		



Key

T2 T2(ms)

repair tissue (light grey box)

control cartilage (dark grey box)

1 repair tissue

2 control cartilage

a 6 months

b 12 months

Figure A.2 — T2 mapping data acquired in ESPREE 1,5T shown as box plots (A) and line pictures (B)

A.3 dGEMRIC evaluation

A.3.1 SKYRA 3,0T (Chinese PLA General Hospital)

The 9 patients at Chinese PLA General Hospital underwent dGEMRIC MR imaging 4 times at 1, 3, 6, and 12 months after MACI using a 3,0 Tesla MRI system (MAGNETOM SKYRA, Siemens AG, Erlangen, Germany) that had a gradient strength of 45 mT/m with a 15-channel phased-array knee coil.

T1 mapping consisted of a sagittal, 3D GRE, volume interpolated breath-hold examination (VIBE) pulse sequence. The sequence was performed both before and after intravenous administration of Gd-DTPA^{2-} (0,2 mM/kg body weight, Magnevist, Schering, Germany). The patient walked for approximately 10-15 min after injection to optimize the distribution of Gd-DTPA^{2-}. Post-contrast T1 mapping was then assessed 120 min after injection until complete diffusion of the contrast agent into the cartilage. The sagittal 3D-VIBE sequence and sagittal FS-PDWI pulse sequences were acquired for precise localization of the regenerative cartilage. Table A.8 summarizes the imaging parameters used in SKYRA 3,0T.

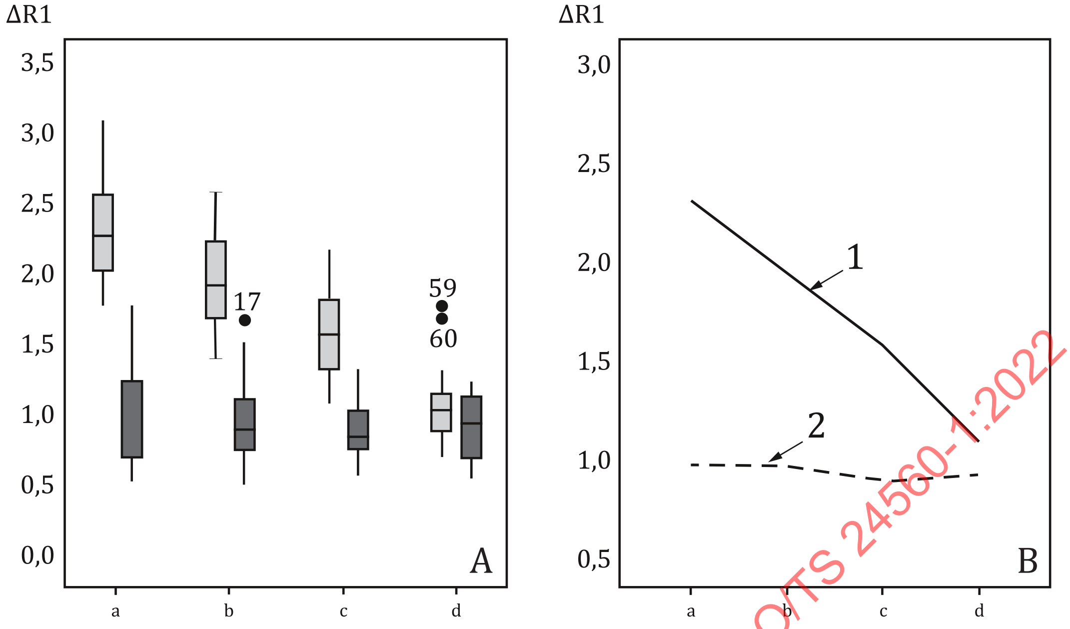
Table A.8 — dGEMRIC parameters in SKYRA 3,0T

	T1 mapping	3D-VIBE	FS-PDWI
FOV (mm × mm)	160 × 160	160 × 160	160 × 160
TR (ms)	15	11,6	3 000
TE (ms)	2,7	5,4	31
Matrix	384 × 384	384 × 384	384 × 384
Flip angles	5; 26	12	none
Voxel size (mm^3)	0,4 × 0,4 × 3,0	0,4 × 0,4 × 0,4	0,4 × 0,4 × 3,0
Number of averages	1	1	1
Slice thickness (mm)	3,0	0,4	3,0
Image plane	Sagittal	Sagittal	Sagittal
Number of slices	24	160	24

Table A.9 — T1 Values (msec) and ΔR1 (1/s) acquired in SKYRA 3.0T

Time (month)	**Repair tissue**			**Control cartilage**		
	T1pre (msec)	**T1post** (msec)	**ΔR1(1/s)**	**T1pre** (msec)	**T1post** (msec)	**ΔR1(1/s)**
1	2 054,02 ± 214,65	360,32 ± 45,01	2,33 ± 0,38	1 159,28 ± 150,31	557,36 ± 101,83	0,98 ± 0,39
3	1 734,47 ± 286,23	398,15 ± 54,04	1,97 ± 0,33	1 135,83 ± 146,16	579,59 ± 84,99	0,97 ± 0,32
6	1 462,78 ± 189,19	447,73 ± 63,87	1,58 ± 0,33	1 084,19 ± 122,41	553,61 ± 67,53	0,89 ± 0,21
12	1 395,36 ± 271,94	555,29 ± 74,21	1,09 ± 0,29	1 135,17 ± 104,39	556,31 ± 65,17	0,93 ± 0,23
P value	< 0,01	< 0,01	< 0,01	0,64	0,99	0,86

NOTE Data are presented as mean values ± SD. *P* values refer to the comparison between 1, 3, 6 and 12 months using Kruskal-Wallis test. R1 = 1 divided by the longitudinal relaxation time.



Key

ΔR1	ΔR1(1/s)
(light grey box)	repair tissue
(dark grey box)	control cartilage
1	repair tissue
2	control cartilage
a	1 month
b	3 months
c	6 months
d	12 months

Figure A.3 — The ΔR1 values acquired in SKYRA 3.0T shown as box plots (A) and line pictures (B)

The T1pre and T1post values of the repaired tissues and the control cartilage at 1, 3, 6, and 12 months after MACI were measured, and the ΔR1 values were calculated (ΔR1= R1post-R1pre, and R1=1/T1). The measurement results showed that the ΔR1 values of the plugs revealed a downward trend with a significant difference at 1, 3, 6, and 12 months after MACI ($P<0,01$). Furthermore, the differences in the ΔR1 and T1post values of the repair tissues and the control cartilage were smaller with a longer follow-up interval. The results are summarized in Table A.9 and Figure A.3.

A.3.2 SPECTRA 3,0T (Peking University Shenzhen Hospital)

The 4 patients at Peking University Shenzhen Hospital underwent dGEMRIC MR imaging 2 times at 3 and 6 months after MACI using a 3,0 Tesla MRI system (Siemens MAGNETOM Spectra) that had a gradient strength of 45 mT/m and with a 15-channel phased-array knee coil.

T1 mapping consisted of a sagittal, 3D GRE, volume interpolated breath-hold examination (VIBE) pulse sequence. The sequence was performed both before and after intravenous administration of Gd-DTPA^{2-} (0,2 mM/kg body weight, Magnevist, Schering, Germany). The patient walked approximately 10-15 min after injection to optimize the distribution of Gd-DTPA^{2-}. Post-contrast T1 mapping was then assessed 120 min after injection until complete diffusion of the contrast agent into the cartilage. The sagittal 3D-VIBE sequence and sagittal FS-PDWI pulse sequences were acquired for precise localization of the regenerative cartilage. Table A.10 summarizes the imaging parameters used in SPECTRA 3,0T.

The T1pre and T1post values of the repaired tissues and the control cartilage at 3 and 6 months after MACI were measured, and the ΔR1 values were calculated (ΔR1= R1post-R1pre, and R1=1/T1). The measurement results showed that the ΔR1 values of the regenerative cartilage exhibited a downward trend with no significant difference 3 and 6 months after MACI (P>0,05). When comparing the repaired tissues and the control cartilage, the ΔR1 values of the plugs were significantly higher than those of the control cartilage at 3 and 6 months (P<0,05). Furthermore, the differences in the ΔR1 and T1post values of the repaired tissues and control cartilage were smaller 6 months after MACI. The results are summarized in Table A.11 and Figure A.4.

Table A.10 — dGEMRIC parameters in SPECTRA 3,0T

	T1 mapping	3D-VIBE	FS-PDWI
FOV (mm × mm)	160 × 160	160 × 160	160 × 160
TR (ms)	15	11,6	3 000
TE (ms)	2,7	5,4	31
Matrix	384 × 384	384 × 384	384 × 384
Flip angles	5; 26	12	none
Voxel size (mm^3)	0,4 × 0,4 × 3,0	0,4 × 0,4 × 0,4	0,4 × 0,4 × 3,0
Number of averages	1	1	1
Slice thickness (mm)	3,0	0,4	3,0
Image plane	Sagittal	Sagittal	Sagittal
Number of slices	24	160	24

Table A.11 — T1 Values (msec) and ΔR1 (1/s) acquired in SPECTRA 3,0T

	T1pre (msec)		**T1post** (msec)		**ΔR1(1/s)**	
	3 months	**6 months**	**3 months**	**6 months**	**3 months**	**6 months**
Control cartilage	1 334,00 ± 116,81	1 342,75 ± 164,64	885,00 ± 42,20	926,50 ± 126,25	0,38 ± 0,09	0,34 ± 0,08
Repair tissue	2 207,75 ± 543,11	1 890,00 ± 316,30	905,25 ± 167,60	926,75 ± 78,45	0,67 ± 0,22	0,55 ± 0,10
P value	< 0,05	< 0,05	0,771	0,995	< 0,05	< 0,01
NOTE Data are presented as mean values ± S.D. *P* values refer to the comparison between control cartilage and repair tissue at 3 and 6 months. R1= 1 divided by the longitudinal relaxation time.						