TECHNICAL SPECIFICATION

ISO/TS 24560-1

First edition

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

PROOF/ÉPREUVE

Reference number
ISO/TS 24560-1:2022(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT),see www.iso.org/iso/foreword.html.

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

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

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].

Delayedgadolinium 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;

