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REVIEW ARTICLE

Functional MRI for evaluation of hyaline cartilage extracelullar matrix, a physiopathological-based approach

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ABSTRACT

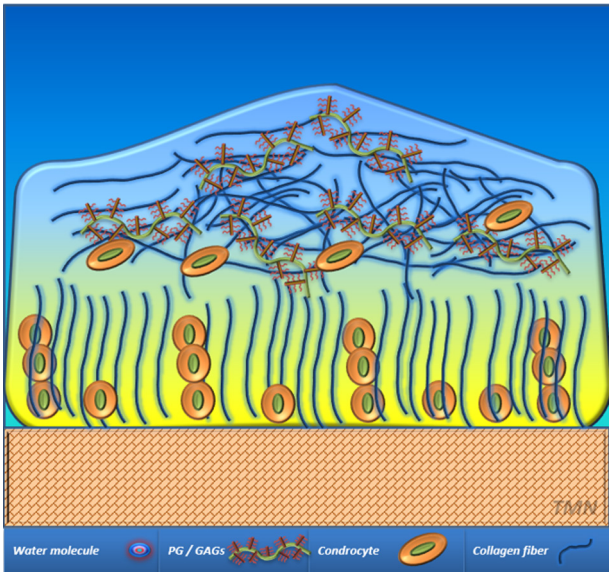
MRI of articular cartilage (AC) integrity has potential to become a biomarker for osteoarthritis progression. Traditional MRI sequences evaluate AC morphology, allowing for the measurement of thickness and its change over time. In the last two decades, more advanced, dedicated MRI cartilage sequences have been developed aiming to assess AC matrix composition non-invasively and detect early changes in cartilage not captured on morphological sequences. T2-mapping and T1ρ sequences can be used to estimate the relaxation times of water inside the AC. These sequences have been introduced into clinical protocols and show promising results for cartilage assessment. Extracelullar matrix can also be assessed using diffusion-weighted imaging and diffusion tensor imaging as the movement of water is limited by the presence of extracellular matrix in AC. Specific techniques for glycosaminoglycans (GAG) evaluation, such as delayed gadolinium enhanced MRI of cartilage or Chemical Exchange Saturation Transfer imaging of GAG, as well as sodium imaging have also shown utility in the detection of AC damage. This manuscript provides an educational update on the physical principles behind advanced AC MRI techniques as well as a comprehensive review of the strengths and weaknesses of each approach. Current clinical applications and potential future applications of these techniques are also discussed.

INTRODUCTION

Osteoarthritis (OA) (*i.e.* degenerative joint disease) is the most common joint disease, with knee OA having a prevalence of 10%–13%.[1] Articular cartilage (AC) changes are seen early in the pathogenesis of degenerative joint disease. Changes in the internal cartilage structure are among the first signs of OA; typically occurring before changes in cartilage thickness.[2] The structure of AC is based on its two main compartments: cellular and extracellular matrix. The cellular compartment is composed by the cells responsible for the generation and preservation of extracellular matrix, the chondrocytes, which represent up to the 10% of the whole volume of normal AC. The extracellular matrix (ECM) compartment is mainly composed of water (up to 70%) and macromolecules, primarily collagen and proteoglycans (PGs)/GAGs (Figure 1).

Clinically, AC is evaluated either indirectly using radiographs or tomographically using MRI, computed tomography or ultrasound.[3,4] Among these techniques, MRI has the highest sensitivity and specificity to detect AC damage and changes in tissue characteristics. Owing to its excelling soft tissue contrast, of the introduction of MRI represented a significant advance in the evaluation of AC allowing for more accurate morphologic characterization. Traditional morphologic MRI techniques have been widely used clinically for quantitative assessment of AC thickness, and can accurately detect focal or diffuse changes in cartilage shape as well as changes in its normal signal intensity.[5] Changes in AC MR signal intensity on morphological sequences such as two-dimensional or three-dimensional T_2-weighted sequences are interpreted as early signs of cartilage degeneration, typically occurring prior to changes in thickness.[6] These signal changes can range from very subtle findings to

Figure 1. AC scheme. The classical layer-based structure of AC has three main zones. The deep zone is characterized by a dominance of collagen fibers that are oriented perpendicular to the surface of AC. Cellular components of AC are commonly identified at this zone, mainly fibroblasts and chondrocytes. The transitional zone usually shows a higher concentration of proteoglycans and glycosaminoglycans that are mixed with randomly orientated collagen fibers and fewer chondrocytes. The superficial zone characteristically shows a predominant orientation of collagen fiber parallel to AC surface in contact with synovial fluid. The line between the deep zona and subchondral bone is called tidemark zone where calcified cartilage can be identified. AC, articular cartilage; GAGs, glycosaminoglycans; PG, proteoglycan.



deeper lesions that extended to the subchondral bone. Changes in signal intensity have been correlated with the degree of cartilage damage with chondromalacia severity staged using semi-quantitative scales.[7] However, morphological MRI sequences lack specificity regarding the underlying pathophysiological changes in AC structure and composition. In most cases, modifications in the cartilage microstructure precede changes in cartilage thickness or signal intensity on morphological MRI sequences. There is a rising interest in the application of a newer MRI techniques to assess cartilage composition and structure.[8–10] These techniques aim to reveal molecular changes to the cartilage ECM composition. With these techniques, focal or diffuse decreases in the collagen or PGs concentration can be assess allowing for an earlier evaluation of cartilage damage or pathology than with morphological MRI techniques.[11] The detection of early changes in cartilage composition is critical to identify individuals at risk of OA progression who can benefit from specific treatment.

The pathophysiological assessment of cartilage structure using MRI techniques can be considered as the next step in comprehensive functional tissue characterization. Toward this aim, several MRI sequences have been developed for visualizing differences in the ECM of normal and abnormal AC. These techniques are based on specific physical or biological differences between normal and abnormal AC that can be assessed or enhanced by using specific technical adjustments to the MRI sequence focused on the different components of the ECM.[12,13] These MRI techniques include T2-mapping, T1ρ, diffusion-weighted imaging (DWI), diffusion tensor imaging (DTI), delayed gadolinium-enhanced MRI cartilage (dGEMRIC), glycosaminoglycan chemical exchange saturation techniques (gagCEST), and sodium imaging.[11,14] The main characteristics of these techniques are summarized in Table 1 and Figure 2.

In this manuscript, we review of these advanced MRI sequences for AC assessment focusing on how each technique evaluates the components of the ECM. In addition, we provide a brief discussion of the current state-of-the-art and clinical use of these techniques.

MRI APPROACHES FOR FUNCTIONAL MRI CARTILAGE EVALUATION

T2-mapping

Physical principle

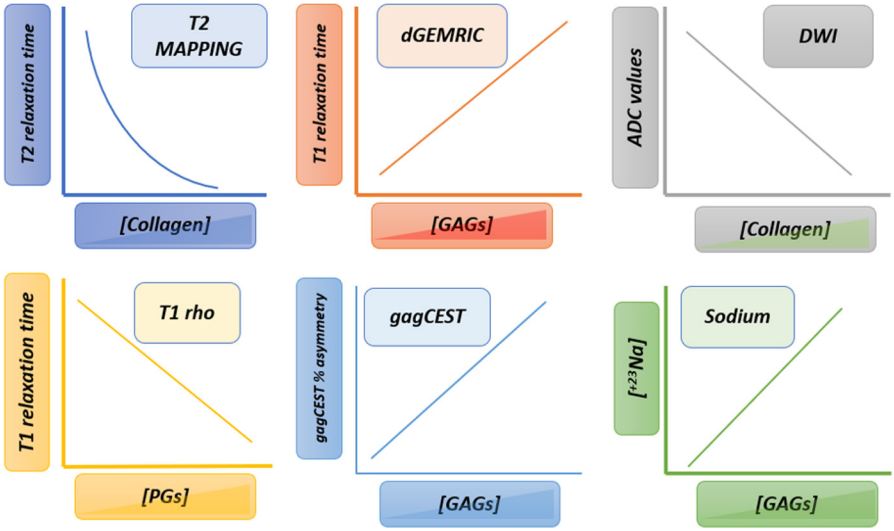
T2-mapping was one of the first quantitative techniques applied to AC evaluation. Sequences for T2 measurement acquire multiple images with varying TE, typically ranging from the lowest values that MRI system allows (~10 ms) to up to over 100 ms.[15,16] A turbo spin echo (TSE) sequence is typically employed. The interaction of ECM, especially collagen fibers, with water protons results in a shortening of T2 relaxation time. Thus, T2 relaxation time depends on the amount of water protons within the cartilage as well as the integrity of ECM, which is mainly

Table 1. Summary of the main functional MRI techniques for cartilage assessment

	ECM target	Gadolinium	Exam time	Clinical use
T2-mapping	Collagen	No	++	++++
T1ρ	PGs	No	+++	+
dGEMRIC	GAGs/PGs	Yes	++++	++
DWI/DTI	Collagen	No	++	+
gagCEST	GAGs	No	+++	+
Sodium imaging	GAGs	No	+++	+/-

DTI, diffusion tensor imaging; DWI, diffusion-weighted imaging; ECM, extracellular matrix; PGs, proteoglycans; dGEMRIC, delayed gadolinium enhanced MRI of cartilage; gagCEST, glycosaminoglycan chemical exchange saturation transfer.
Symbols range from (-) short exam time/limited clinical use to (+) large exam time/extended clinical use.

Figure 2. Summary techniques. Each functional technique is able to assess, in dominant manner, the integrity or depletion of a specific component of ECM. Thus, the relationship between collagen fiber concentration and T2 relaxation time is inverse, as well as the relationship between ADC values and collagen fibers. PGs and GAGs concentration have a positive correlation with the T1 relaxation times after gadolinium injection in dGEMRIC model as well as a positive lineal correlation with the percentage of gagCEST asymmetry and sodium concentration. T1 relaxation time in T1rho acquisition has inverse correlation with PGs concentration. ADC, apparent diffusion coefficient; dGEMRIC, delayedgadolinium enhanced MRI of cartilage; ECM, extracellular matrix; GAG, glycosaminoglycan; gagCEST, Chemical ExchangeSaturation Transfer imaging of GAG; PGs, proteoglycans.



reflected by collagen fiber density. A direct correlation between T2 values and water content and an inverse correlation with collagen concentration within AC has been shown.[17] Although color T2 maps can be directly visualized for qualitative interpretations, there are dedicated software packages available to extract the quantitative information; some of these packages permit semi-automated segmentation of the region of interest.Supplementary Video 1 .

Ex vivo/in vivo validation

Several studies have demonstrated that collagenase-degraded cartilage samples have a higher T2 value than healthy cartilage due to the loss of normal ECM composition and loss of collagen integrity and concentration.[18,19] The disruption of the matrix decreases the collagen concentration and increases the overall water content within AC; both factors contribute to higher T2 relaxation times.[19] While T2-mapping is influenced by loss of all ECM, including GAGs (and PGs), most of studies have demonstrated a greater specificity for collagen fiber assessment.

T2 values are less reliable in the deep and calcified cartilage layers, which in most cases are only evaluated by using ultrashort TE approaches.[20,21] In addition, there is some spatial variation with superficial layers of AC having higher T2 relaxation times than deeper layers.[18] T2 values may also be affected by the magic angle effect, which may introduce regional variation based collagen fiber orientation and can make longitudinal comparisons challenging.[22] T2-mapping studies do not require high magnetic fields and thus can performed at 1.5 and 3 T. An additional benefit is that T2-mapping does not require administration of exogenous contrast agents.

Clinical value

T2 mapping has been applied for evaluation of OA with promising results. Focal or diffuse areas of increased T2 relaxation times have been identified in patients with OA even prior to changes on morphological sequences Figure 3. Moreover, a positive correlation between T2 values and cartilage damage has been demonstrated. As T2-mapping is less sensitive to PGs

Figure 3. T2-mapping cartilage assessment. A 32-year-old female runner with knee pain. (a) A focal area of signal intensity increase is identified (arrow) at trochlea on morphological axial FFE T_2W sequence. (b) T2-mapping confirms a focal defect of signal with severe increase of T2 relaxation times at trochlear sulcus as well as early cartilage damage changes at superficial cartilage layer (white arrow), which not clearly seen on morphological sequence. FFE, fast field echo; T_2W, T_2 weighted.

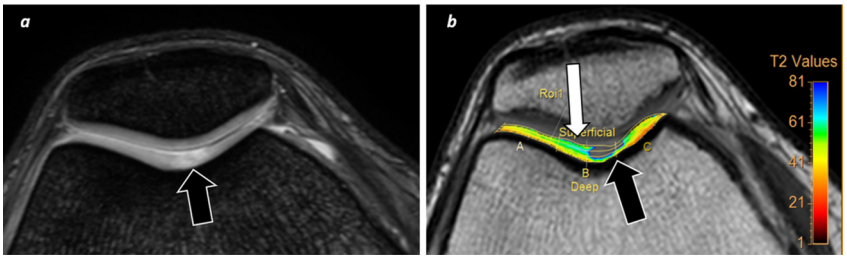
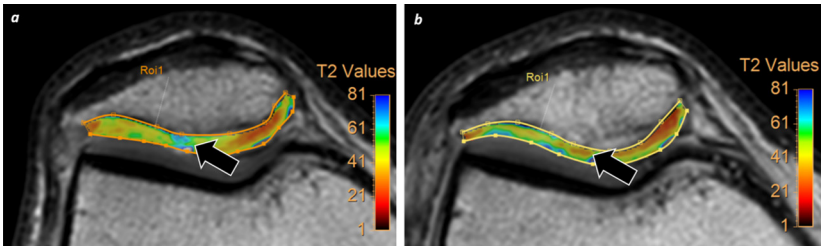


Figure 4. Treatment monitoring. A 46-year-old male with knee pain. (a) First study was performed on January 2018 whereas a focal area of increase of T2 relaxation values was identified at lateral aspect of articular surface of patella (white arrow) on T2-mapping sequences consistent with chondromalacia. Patient undergone surgery (chondrocyte graft) and a new MRI was performed on March 2019. (b) This new study shows decrease of T2 values at surgical area (white arrow) which suggest presence of new hyaline cartilage.



concentration, it can provide complementary information to other techniques such as dGEMRIC, sodium imaging or T1ρ mapping that are mainly affected by the loss of GAGs/PGs. T2-mapping has also been used for follow up after knee cartilage repair surgery. These studies have shown decreased T2 relaxation times of repair tissue after microfracture compared to healthy cartilage, likely owing to its more fibrocartilaginous construct.[23] However, on longer term follow-up in patient treated with microfracture, T2 relaxation values in the cartilage repair zone tend to normalize with respect to the rest of cartilage.[24] Other repair approaches such as chondrocyte grafts, tend to form hyaline-like cartilage showing T2 relaxation values similar to healthy AC (Figure 4).[25]

T1ρ

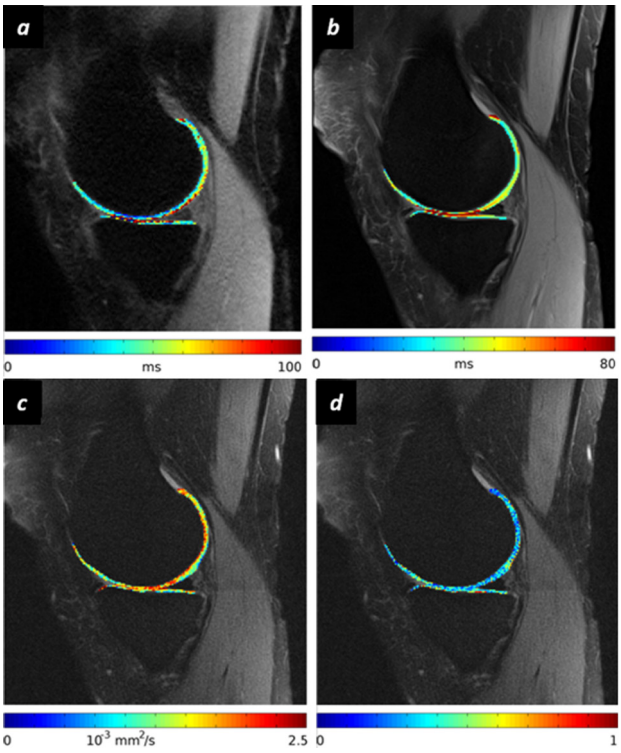
Physical principle

T1ρ imaging assesses the relaxation of spin under the influence of a constant radiofrequency (RF) pulse that is set just when the magnetization is tipped into the transverse plane. The role of this RF pulse is to spin-lock the magnetization. T1ρ is the constant that reflects the spin-lattice relaxation time in the rotating frame after application of the RF spin-lock pulse as there is a relationship between T1ρ and the interchange of energy between water molecules and macro-molecules.[26,27] Several studies have demonstrated that T1ρ values depend on the proton exchange with amide and hydroxyl groups present on the PGs/GAGs side-chains.[28,29] Thus, the slow interaction of water molecules and ECM can be accurately assessed in a way similar to the interaction of water molecules and collagen in T2-mapping sequences.[17] This technique does not require gadolinium-based contrast agents (GBCAs). However, T1ρ mapping does require an MRI system able to create a specific RF pulse for achieve a proper spin-locking. The time required for T1ρ acquistition is considerably longer than for T2-mapping (Supplementary Video 2[10,30],Figure 5a and b).

Ex vivo/in vivo validation

The evaluation of T1ρ imaging using *ex vivo* models allows for the assessment of variations in PGs concentration in AC. The use of trypsin enzymes for PGs degradation in bovine articular specimens demonstrated that the T1ρ approach was more sensitive for detection of changes in PGs concentration than conventional T_1 or T_2 weighted images.[31] After collagenases administration, these studies did not reveal significant sensitivity of T1ρ for collagen depletion assessment within AC.[32,33] *Ex vivo* human specimens have demonstrated similar results showing that the dominant contribution to T1ρ AC imaging is from PGs degradation rather than collagen degradation.[34]

Figure 5. Examples of MRI parameter acquisition on a 54-year-old subject with knee OA (KL Grade 1). (a) T1ρ map of cartilage acquired with a spin lock frequency of 500 Hz and spin lock times of 10, 20, 40, 60 ms. Background image corresponds to 10 ms. (b) T2 parameter map of cartilage acquired with a multiecho turbo spin echo sequence (TEs from 10 to 100 ms). (c, d) Diffusion tensor imaging of the knee (RAISED pulse sequence). Diffusion images are acquired with a *b*-value of 300 s/mm^2 and a TE of 35 ms. MD (c) and FA (d) overlaid over the b0-image. FA, fractional anisotropy; MD, mean diffusivity; OA, osteoarthritis; RAISED, radial imaging spin echo diffusion; TE, echo time.



Clinical value

T1ρ is able to detect changes in the composition of ECM related to PGs concentration as occurs in osteoarthritis. T1ρ values are higher in healthy volunteers. In the early stages of the OA, T1ρ is more accurate than T2-mapping.[35] Moreover, T1ρ values are able to discriminate between intermediate and advanced cartilage degeneration. However, since T2-mapping and T1ρ evaluate two different relaxation mechanisms within cartilage, the information provided should be considered complementary, not exclusionary.[36] T1ρ has also been applied for assessment of early cartilage degeneration in patients with meniscal lesions. The loss of PGs in the femoral condyles in these patients correlates with increase of T1ρ values.[28] Patient with anterior cruciate ligament injuries, even years after reconstruction, may also show changes in AC composition that can be accurately assessed by T1ρ.[37,38] T1ρ has been measured in conjunction with synovial fluid biomarkers such as GAGs concentration in order to evaluate, in a non-invasive manner, the characteristics and integrity of ECM. These studies show a negative correlation between T1ρ relaxation times and GAGs concentration in both cartilage and synovial fluid.[27,36]

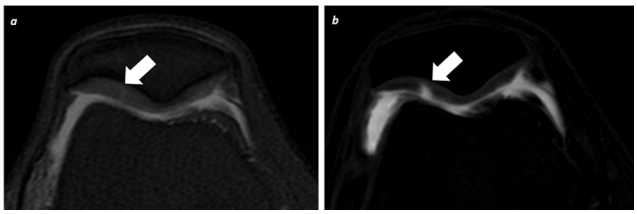
Physical principle

dGEMRIC is a non-invasive functional technique to study cartilage GAGs content *in vivo*. The dGEMRIC technique uses the negatively charged contrast agent gadolinium-diethylene triamine pentaacetic acid (Gd-DTPA^{2-}).[39] GBCAs can be administrated either intra-articularly or intravenously. However, the intravenous injection (usually with double dose) is preferred over the intra-articular injection as there is low risk of septic arthritis and the higher rate of gadolinium penetration (from both articular surface and subchondral bone).[40] After intravenous injection and systemic circulation, Gd-DTPA^{2-} distributes within cartilage in a manner inversely related to the negatively charged GAGs content. Gd-DTPA^{2-} shortens the T1 relaxation time of cartilage. In addition, the need for exogenous contrast agent administration, the dGEMRIC technique has other drawbacks. A delay of over 60 min after gadolinium administration is mandatory to ensure its filtration through the synovial membrane into synovial fluid and its diffusion into the cartilage. Moreover, a specific MRI sequence with multiple inversion times (TIs) has to be acquired for a proper quantification of the T1 shortening of the cartilage in a manner similar to the T1-mapping technique. An intermediate approach is a semi-quantitative assessment by acquiring heavily weighted T1 sequences that allow the radiologist to evaluate the presence or absence of gadolinium uptake at AC Figure 6. Long T1 relaxation time values are found in healthy cartilage whereas short T1 relaxation times values are found in degenerated cartilage due to the high amount of infiltrated Gd-DTPA^{2-} (Supplementary Video 3).

Ex vivo/in vivo validation

The dGEMRIC approach has been tested using *in vitro* and *ex vivo* models with robust results for both animal and human AC assessment.[41] *In vivo* and *ex vivo* studies for GAGs concentration and distribution within AC have demonstrated good histological and biochemical correlation.[42] These kinds of studies have been necessary in order to determine the type of GBCA needed to obtain the highest tissue contrast within AC. In this respect, there are studies with contradictory results with regard the penetration rate of non-ionic GBCAs and ionic GBCAs, however, the global recommendation is to use ionic GBCAs.[39]

Figure 6. dGEMRIC assessment. A 41-year-old male tennis player, who refers knee pain with flexion and extension movements. (a) Conventional axial FFE T_2W shows non-significative increase of signal intensity at lateral patellar articular surface (arrow). (b) Qualitative dGEMRIC sequence after intraarticular gadolinium injection, demonstrates intense uptake of gadolinium at this area (arrow) consistent with loss of GAGs (focal lineal ulcer with deep cartilage involvement). dGEMRIC, delayed gadolinium enhanced MRI of cartilage; FFE, fast field echo; GAG, glycosaminoglycan; T_2W, T_2 weighted.



Clinical value

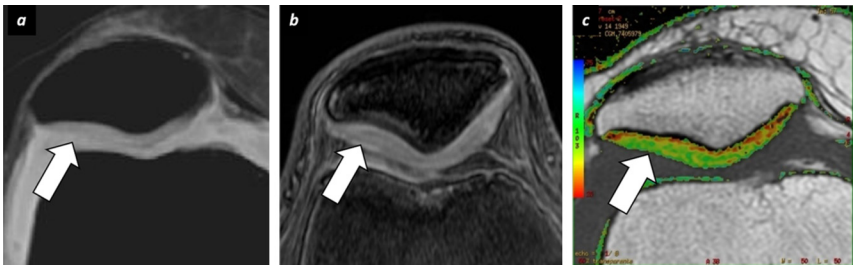
The dGEMRIC technique is a useful clinical imaging method for evaluating cartilage biochemical composition and to monitor the effects of therapies for osteoarthritis and cartilage injury. Those areas with GAGs concentration preserved will not uptake gadolinium as the negative charged hydroxyl group prevents significant gadolinium diffusion into cartilage (Figure 7). On the other hand, those areas with loss of GAGs (and their negative charges) will allow gadolinium molecules to penetrate the cartilage surface. Several studies have demonstrated the utility of the dGEMRIC approach for detecting early signs of OA related to the loss of GAGs in knee.[43] In these patients, lower T1 relaxation times are identified in areas with cartilage damage compared with areas of normal cartilage. dGEMRIC has also be applied in other anatomical regions and with different purposes than evaluation of OA. For example, in hip dysplasia, dGEMRIC has been tested with good correlation with patient symptoms and the severity of dysplasia.[44]

DWI/DTI

Physical principle

Water molecules in biological tissues are in constant and random motion due to the thermal energy and interactions with macromolecules or membranes.[45] In DWI, the microscopic random displacements of water molecules is measured to extract information about tissues.[46] DWI measures the average displacements of all water molecules in a voxel along a direction (diffusion direction) during a time called diffusion time.[47] Using the measurements of average water displacement in different directions, we can infer the organization of the underlying tissue microstructure using diffusion models. The ECM of AC constrains free diffusion of water molecules. DWI allows one to estimate the integrity of ECM based on the motion of water molecules.[48] The apparent diffusion coefficient (ADC) can be used to measure the average movement of these water molecules in mm^2/s (Supplementary Video 4).[49] AC has a water content of approximately 75% and

Figure 7. Multimodality functional MRI cartilage evaluation. A 29-year-old male which refers knee pain after running. (a) Axial fat suppression PD sequence shows slight increase of signal intensity within the lateral aspect of patellar cartilage (arrow). (b) Qualitative assessment of axial dGEMRIC acquisition does not reveal any abnormal gadolinium uptake at this area (arrow). (c) Axial T2-mapping shows increase of T2 relaxation time values at this level (arrow). The combination of findings described suggest hyaline cartilage damage due to loss of collagen fibers with intact GAGs levels. dGEMRIC, delayed gadolinium enhanced MRI of cartilage; GAG, glycosaminoglycan.



physiologically displays ADC values in the range between 1.4 and 1.6×10^{-3} mm^2/s.[6] Cartilage damage results in less restriction to the motion of water and thus increased ADC values (Figure 8).

To extract information about the tissue structure we measure the diffusion in at least six non-collinear directions and use the diffusion tensor model.[50] This model describes the diffusion in tissue as a symmetric tensor. Through measurements of diffusions in at least six non-collinear directions, all elements of the tensor can be calculated. Parameters derived from DTI such as fractional anisotropy (FA), which is an index of tissue organization, or the mean diffusivity (MD), which represents the average of the main three eigenvalues (the numeric representation of the magnitude of each direction of water molecules within the tissue). Applied to cartilage, these parameters provide a method to differentiate the contributions of collagen structure, as captured with the fractional anisotropy, from the PGs as captured with the mean diffusivity (Supplementary Video 5).[49]

Ex vivo/in vivo validation

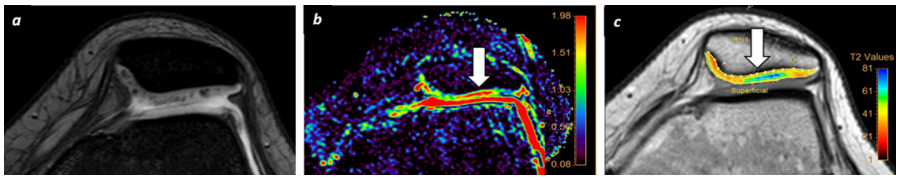
Ex vivo studies have reported a 5—30% increase of diffusivity after enzymatic depletion of PGs that correlated with GAGs concentration.[48,51–53] Diffusivity also increases with disease severity in osteochondral samples.[54,55] Enzymatic cleavage of PGs resulted in increased MD but resulted in no change in FA or diffusion orientation, thus indicating the ability of DTI to track both collagen and PGs changes independently.[56,57] MD has shown correlation with mechanical properties of cartilage.[58] MD and FA also changed with OA.[59] Indeed, MD and FA have shown the potential to grade histologic cartilage damage as measured with the Osteoarthritis Research Society International score with an area under the receiver operating curve between 0.7 and 0.9 for the detection of early degenerative changes (Figure 5C and d).[59,60]

Clinical diagnostic and prognostic value

Translation of diffusion measurements to the clinical scanners was hampered by the technical challenges of acquiring diffusion in a low-T2 (~30 ms) tissue with high resolution (<1 mm). Clinical echoplanar imaging sequences have not been able to provide satisfactory results due to their long TEs (>80 ms), their sensitivity to susceptibility artifacts, and their limited resolution (~2–3 mm).[61] Thus, the first studies used only diffusion-weighted images with steady-state free precession sequences assessing changes semi-quantitatively.[62] Spin-echo-based sequences provide excellent image quality.[63,64] The first clinical studies of DTI were published at 7 T using a line scan sequence and showed potential to differentiate the patellar cartilage of healthy and early OA subjects (area under the receiver operating curve = 0.92, n = 26).[63] DTI studies have shown significant increase in MD (10 to 20%) between healthy controls and OA subjects and a decrease in FA (−18 to −11%), both at 3 and 7 T. Reproducibility of DTI *in vivo* was high with variation below 4% for MD and 6% for FA on repeated measurements.[63,65]

In summary, diffusion imaging can provide insight in cartilage microstructure. DTI has the potential to track changes in both collagen and PGs. Clinical studies are still sparse but have

Figure 8. Evaluation of AC with DWI in a 45-year-old male with knee instability. (a) Conventional axial FFE T_2W sequence does not show clear abnormalities at patellar cartilage surface. (b) DWI acquisition demonstrates increase of ADC values at lateral patellar surface (arrow) revealing loss of collagen fibers. (c) T2- mapping shows proper correlation with DWI demonstrating also increase of T2 values at the same area (arrow). Both functional techniques reflect collagen loss with different physical basis and show early cartilage damage changes before morphological imaging. AC, articular cartilage; ADC, apparent diffusioncoefficient; DWI, diffusion-weighted imaging; FFE, fast field echo; T_2W, T_2 weighted.



consistently shown feasibility of these techniques and their potential for early diagnosis.

gagCEST

Physical principle

gagCEST can be considered a technical upgrade of conventional magnetization transfer (MT) sequences. MT allows for the evaluation of the contribution to MRI signal from the protons in unbound bulk water molecules that are present in certain tissues. The final goal of the MT, and gagCEST, techniques is to increase the contrast between free water and bound water, evaluating the exchange of energy between these two water pools. In the case of gagCEST, a selective RF pulse excites exchangeable GAGs protons.[66] These excited protons experience a chemical exchange phenomenon with free water protons that results in a quantifiable decrease in the magnetization of the free water pool. The target in the case of GAGs is the hydroxyl and sulfate group, with allows one to measure the GAGs concentration *in vivo* within AC. The amount of the magnetization transfer between both water pools (free and bound to GAGs) can be expressed as the asymmetry of magnetization transfer ratio (MTRasym), which reflects the distribution of free protons around a central water peak in AC. This parameter is obtained after saturating protons linked to GAGs on either side-of the water peak. As occurs with other techniques that require the application of selective RF pulses, a high strength magnetic field (up to 3 T) and very homogeneous B0, are needed to saturate of the hydroxyl group, which has a resonance frequency very close to that of free water (Supplementary Video 6).

Ex vivo/in vivo validation

Several studies have evaluated the physical principle of chemical exchange saturation in *ex vivo* models (animal and phantoms) confirming the feasibility of this approach to characterize AC damage and for early damage detection. Phantom studies are required in order to properly adjust the RF pulse to GAGs exchangeable protons saturation as well as to center the CEST spectrum and fitting it with water resonance frequency. The fitting of the whole spectrum of gagCEST is time consuming in presence of B_0 inhomogeneities, so strategies such as the acquisition of a dual gradient echo B_0 map have been proposed to reduce scan time.[67] Regarding the need for high magnetic field systems, some authors have evaluated the differences in gagCEST asymmetry *in vivo* between 3 and 7 T. After B_0 inhomogeneities correction, the gagCEST asymmetry at 3 T is scarce, so 7 T is recommended in order to increase gagCEST asymmetry and thus, tissue contrast between damaged and healthy AC.[68]

Clinical diagnostic and prognostic value

Currently, the application of gagCEST techniques for AC assessment is uncommon in clinical practice due to the technical requisites described above. Nevertheless, several studies have evaluated the potential applications of this approach for AC assessment.[69] In these studies, higher MTRasym values have been detected in healthy volunteers than in patients with OA reflecting, in a very specific manner, a loss of GAGs from cartilage even in early stages.[70] Some authors have even compared the accuracy of dGEMRIC and gagCEST, both of which are able to assess loss of GAGs with similar results. For cartilage repair assessment or even the introduction of new therapies in the firsts stages of OA, the gagCEST approach has shown promising results in patient monitoring; showing normalization of MTRasym, which suggests recovery of GAGs concentration within AC.[71]

Sodium imaging

Physical principle

Most MRI systems are designed for the detection and characterization of ^{1}H protons. However, there are other compounds within the tissues, such as sodium which can also be evaluated. ^{+23}Na is an ion characterized by a quadrupolar moment able to interact with surrounding protons, which condition a biexponential decay of relaxation times.[72] ^{+23}Na (positively charged) and PGs (negatively charged) establish an electromagnetic equilibrium inside the AC with a direct correlation between both compounds. Sulfate and carboxylate PGs groups are fixed to ^{+23}Na, so a loss of PGs involves a decrease of ^{+23}Na concentration within cartilage.[8] However, SI of AC has two major drawbacks: its very low concentration and its very short transverse relaxation time. These disadvantages result in poor quality images due to low SNR, poor resolution and partial volume effects from synovial fluid and subchondral bone.[73] To try and overcome these limitations, and increase the resolution and SNR of sodium acquisitions, the use of dedicated coils with long scan times and high magnetic field systems is mandatory (Supplementary Video 7).[74,75]

Ex vivo/in vivo validation

In vivo and *ex vivo* studies have demonstrated the existence of a fixed charge density (FCD) within AC due to a balance between ^{+23}Na and GAGs. That FCD enables the calculation of the GAGs concentration in the AC. Animal studies with bovine cartilage and PGs damage induced by trypsin demonstrate changes in sodium concentration with an almost linearly relationship.[76] Sodium MRI has also been applied for OA evaluation in experimental animal models after cytokine injection; detecting a decrease of sodium concentration as well as FCD. The results are consistent with loss of PGs and these studies have shown histological correlation.[29]

Clinical diagnostic and prognostic value

The use of sodium MRI in common clinical practice is quite limited due to the drawbacks listed above. However, some studies have tested the feasibility of sodium MRI for cartilage evaluation in healthy volunteers and patients with early signs of OA.[77] Lower signal in the sodium image, consistent with loss of fixed charge density, has been identified in patients with knee OA compared with healthy controls, which suggests a decrease in PGs concentration within AC.[75] Nevertheless, no large series have yet been published evaluating the clinical impact of this approach for assessment of AC damage. Further studies are needed to establish the potential role of this promising tool as well as the sensitivity and specificity with regard the rest of functional imaging modalities for cartilage assessment.

CONCLUSIONS

Currently, there is a wide range of functional MRI techniques that are allowing radiologist to evaluate AC in a more

accurate manner than with morphological MRI sequences alone. These techniques predominantly evaluate the integrity of each one of AC components, mainly collagen and GAGs/PGs. T2-mapping can be considered the most robust and ready for practical use in current clinical radiology practice due to its relative short acquisition time and its almost worldwide implementation. Other approaches, such as dGEMRIC, also used in clinical practice, have the major drawback of the need for GBCAs administration. To this point, non-contrast techniques like DWI or DTI may become viable alternatives. T1ρ, gagCEST and Sodium imaging have also show high specificity for GAGs/PGs assessment but they are used uncommonly in clinical practice due to the longer acquisition times and higher magnetic fields strength requirement. Nevertheless, the knowledge of the physical basis of how each technique allows one to evaluate ECM in a specific manner is important for radiologists. Being familiar with these techniques will allow physicists and radiologists to optimize the acquisition and interpretation of these studies, not only for investigational purposes but also for their application in current and future clinical radiologic practice.

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