

T2 mapping: an efficient MR quantitative technique to evaluate spontaneous cartilage repair in rat patella¹

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Summary

Objective: to evaluate the ability of T2 mapping on an 8.5T imager to characterize morphologically and quantitatively spontaneous repair of rat patellar cartilage following full thickness defect.

Methods: Patellar cartilage defects were created in 24 rats knees on D0. Eight rats per time-point were killed on D20, D40 and D60 after surgery. T2 maps of repair tissue in patellar defects were obtained from eight different axial spin echo images on an 8.5T imager. Global, superficial and deep T2 values were evaluated in spontaneous repair tissues (3×8 right patellae) vs the opposite patellae (3×8 left patellae) of the same animals. MR data were compared with macroscopic and histological studies.

Results: T2 map was able to identify morphologically three types of repair tissue observed macroscopically and histologically: 'total', 'partial' and 'hypertrophic' repair tissue. 'Total' and 'partial' repair tissues were characterized by global T2 values almost similar to controls, whereas 'hypertrophic' repair tissues were characterized by T2 global values higher than controls. Zonal variation between superficial and deep T2 values observed in controls was not depicted in repair tissue before D60.

Conclusion: T2 map is able to characterize quantitatively and qualitatively rat patellar cartilage repair, and thus can be promoted, as a non invasive technique, in clinical longitudinal studies of articular cartilage repair.

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Key words: Magnetic Resonance Imaging, T2 mapping, Cartilage repair.

Introduction

Articular cartilage is a nonvascularized tissue where chondrocytes are surrounded by an extracellular matrix composed of two major constituents: proteoglycan, responsible for hydration and deformity, and collagen, responsible for stiffness. The ability of cartilage to be repaired is weak, and leads to a fibro-cartilaginous tissue, with poor biochemical and biophysical qualities in comparison to hyaline cartilage. Osteoarthritis (OA) is mainly observed in older population and is usually secondary to a large breakdown of the cartilage, often accompanied by subchondral bone modifications and synovial inflammation. In some cases,

OA may also be secondary to focal cartilage lesions, especially in young sportive patients, related or not to direct trauma, and actually treated by either mosaicplasty¹ or autologous chondrocyte grafts². Such focal lesions are not depicted on X-rays since the joint space is preserved. Thus, to date in clinical practice, the diagnosis of these lesions is made by arthroscopy which remains the gold standard method to depict and to evaluate cartilage lesions according to their severity, surface and topography^{3,4}. However, this invasive procedure cannot be repeated for a longitudinal study over a short time-period.

Conversely, MRI is a non traumatic technique which also permits a direct evaluation of chondral lesions according to their severity, surface and topography⁵. This technique also offers the opportunity to follow the progression of cartilage lesions, to depict subchondral and trabecular bone modifications, synovial inflammation, and to evaluate surgical treatment (cartilage grafts). Recently, Alparslan *et al.*⁶ demonstrated that MRI permits to follow surgical graft according to the mosaicplasty technique in focal cartilage lesions in human knee and to differentiate normal from abnormal tissue repairs (partial repair, hypertrophic repair, delaminated and adhesion patterns). Additionally, unlike arthroscopic examination, MRI permits to evaluate the

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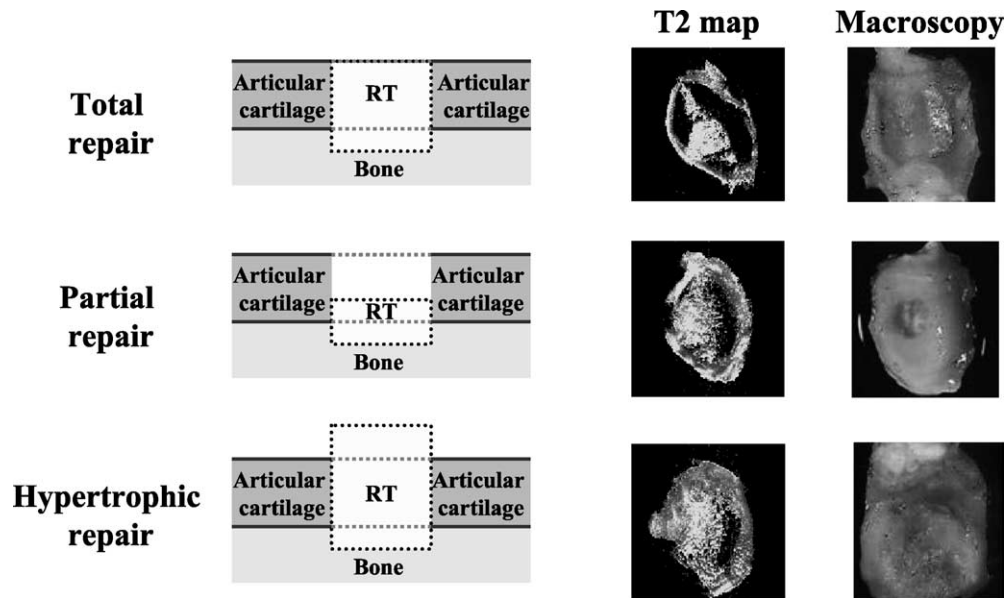


Fig. 1. MR and morphological patterns of the three types of repair tissue (RT) observed after full-thickness defect of the patellar cartilage. 'Total' repair was defined by a superficial limit of the defect located at the same level as the adjacent cartilage. In 'partial' repair, the superior border corresponded to a virtual line joining the adjacent cartilage. 'Hypertrophic' repair is characterized by a voluminous tissue formation localized over the adjacent cartilage. The T2 map is able to characterize these different types of repair tissue.

internal structure of cartilage (edema or fibrosis) and also to explore the cartilage bone interface.

Beyond the morphological evaluation, MRI appears as a promising technique to characterize the structural organization (collagen network) and the matrix content of the cartilage⁷. Such a quantitative MRI structural approach may be performed in clinical practice either by T1 mapping technique plus contrast agent or by T2 mapping technique. Recently, Gillis *et al.*⁸, demonstrated the ability of T1 mapping technique plus contrast agents to quantitatively evaluate the proteoglycan content at 2, 6, 12, 18, and 24 months after autologous chondrocyte grafts in 10 patients suffering from focal cartilage lesions. T2 mapping technique had recently shown its potentiality to evaluate the quality of the cartilage composition in human^{9–11} and animal cartilages¹² since the transversal relaxation time (T2) reflects the ability of proton molecule to move and to exchange energy inside the cartilaginous matrix^{13–15}. Each variation of matrix content (water, proteoglycan and collagen) or each modification of the organization of the collagen network can induce T2 variations. Thus, we have previously demonstrated that T2 mapping was able to quantify matrix content during maturation process and also to depict proteoglycan loss in rat patellar cartilage¹⁶. The aim of this study was to evaluate the ability of T2 mapping, performed on a 8.5T imager, to characterize morphologically and quantitatively the spontaneous cartilaginous repair process in rat patellar cartilage at various time points after creating a cartilage lesion. Three quantitative parameters were evaluated in normal cartilage and in spontaneous repair tissues: global, superficial and deep T2 values. Global T2 values obtained from all cartilages characterized the matrix content while zonal variation of T2 values between superficial and deep zones appreciated its structural organization. In addition, T2 mapping data (morphological and quantitative parameters) were compared to macroscopic and microscopic parameters.

Materials and methods

ANIMALS

Twenty-four male Wistar rats (48 patellas) underwent a surgical defect of their right patellar articular cartilage and were killed and studied at three different time-points of repair: D20 ($N=8$), D40 ($N=8$), and D60 ($N=8$). Left knees were not operated and served as controls to avoid variations due to maturation process [Fig. 1].

SURGICAL PROCEDURE

Rats were anesthetized by an intraperitoneal injection of a mixture of acepromazine (1 mg/kg) and ketamine (38 mg/kg). Under rigorous aseptic conditions, a medial parapatellar incision was made and the patella was reverted through 180° to expose its articular surface. A 1.3 mm wide defect was drilled in the middle part of the patella from its surface until the subchondral bone. The patella was then repositioned and the periarticular muscles were repaired with Ethicon Vicryl® (Johnson & Johnson Intl, Belgium) sutures and the skin closed with Polyamide Filapeau® (Peters, France) sutures. The limb was not immobilized and the animals were allowed free-cage activity. The maintenance and care were respected as mentioned in the National Institutes of Health guidelines. At each time (D20, D40 and D60), animals were anesthetized and killed by cervical dislocation, knees were carefully dissected and kept frozen until MR study since preliminary studies showed that this procedure did not alter MR signal.

MR IMAGING AND T2 MAPPING OF RAT PATELLAR CARTILAGE

The thawed patellae were dissected from the knee just before MR Imaging exploration and placed for 16 h at 4°C in saline solution to correct the frozen process-related

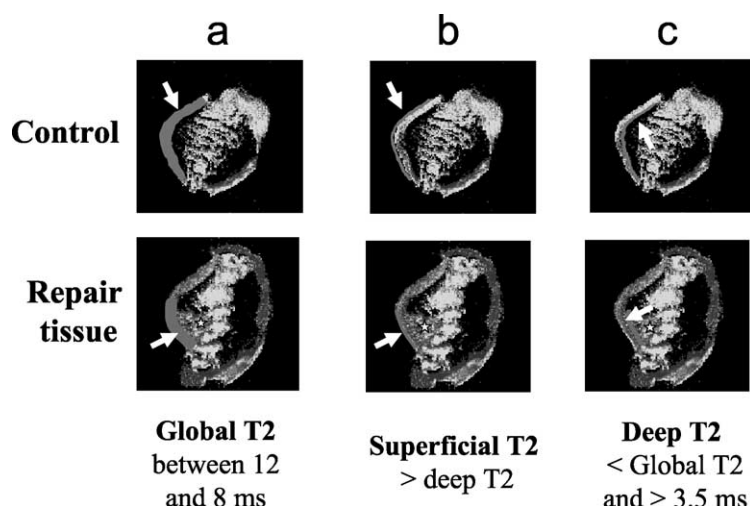


Fig. 2. Measurement of global, superficial and deep T2 values. (a) The global T2 value was calculated as the mean of T2 values of cartilage including the superior and the inferior borders. The superficial (b) and deep (c) T2 values corresponded respectively to the mean of the values of the superior border and of the values of the inferior border.

dehydration. Patellae were put into a closed atmosphere in a sample tube. All images were obtained with an 8.5-T MR micro-imager (Oxford instrument, Osney Head, England) with identical experimental parameters. Previously, a sagittal acquisition was done in order to localize the repair tissue. Then, sequence of T2 mapping was acquired in a transversal plane through the repair tissue zone. All the patellae were oriented in the same direction in the sample tube placed inside the coil, with the superficial collagen fibers of patellar cartilage oriented perpendicular to the magnetic field strength. Eight spin echo images were obtained with the following parameters: constant repetition time of 1.5 s; eight different echo times (5.5; 7.5; 10.5; 12.5; 15; 20; 25 and 30 ms); four signals acquired; total acquisition time of 105 min. The section thickness was 1 mm, the field of view was 4x4 mm, the matrix size was 128x128, and the spatial resolution was 31x31x1000 μm^3 . During MR imaging acquisition, the magnet bore temperature varied between 25 and 30°C.

T2 values were calculated from the eight spin-echo images by using nonlinear least squares curve fitting on a pixel-by-pixel basis. The signal intensity, SI , for the i th j th pixel as a function of time (t), can be expressed as follows: $SI_{ij}(t) = SO_{ij} \exp(-t/T2_{ij})$, where SO_{ij} is the pixel intensity at $t=0$, and $T2_{ij}$ is the T2 time constant of the pixel ij . The T2 map was displayed on a pixel-by-pixel basis (Transform; Spyglass, Savoy, Ill) for the cartilage itself but also for adjacent structures such as subchondral and trabecular bones. Data can be represented on a color scale; they were stored as 2-byte integers covering the range 0–32.767 ms, adapted to the cartilage T2-values observed at 8.5T.

Morphological analysis of T2 map

In normal cartilage, the superficial border of the cartilage was easily defined owing to the large difference in T2 values between air and cartilage. Conversely, both calcified cartilage and subchondral bone had low T2 values, which did not permit a clear delineation between both tissues.

With this in mind, on T2 maps, the value of 4 ms was considered to be the lower threshold value for the deepest part of the noncalcified tissue.

In patellar cartilage repairs, three types of repaired tissue may be observed: 'partial', 'total' and 'hypertrophic' repairs [Fig. 1]. On T2 mapping, these repair tissues were also delimited by superior and inferior borders.

- In 'partial repairs', the superior border corresponded to a virtual line joining the superficial limits of the adjacent cartilage. The superficial limit of the repaired tissue was thus placed under the superior border.
- In 'total repairs', the superior border corresponded to the superficial limit of the repaired tissue which was located at the same level as that of the adjacent superficial cartilage.
- The 'hypertrophic repairs' were characterized by a voluminous tissue formation localized over the adjacent cartilage, covering in some cases its surface. The superior border corresponded to the superficial limit of the hypertrophic tissue.

In all repairs, the inferior border was not clearly identified and corresponded to a virtual line joining the adjacent subchondral bone.

Quantitative analysis of T2 map

The T2 global values [Fig. 2 (a)] of normal rat patellar cartilage and repair tissues were calculated as the mean of pixel values between these two borders (superior and inferior borders). Superficial and deep T2 values [Fig. 2 (b) & 2(c)] corresponded to the mean values of pixels that constituted the superior and inferior borders respectively. Thus, zonal variations in T2 values were appreciated as the difference between superficial and deep T2 values. Immediately after MRI exploration, patellae were fixed in formalin solution before macroscopic and microscopic analysis.

Table I
Macroscopic score used for the evaluation of the quality of the repair tissue according to 5 parameters

Parameters	Observations	Score*
Global aspect of the repair zone in comparison to normal cartilaginous tissue	Identical	2
	Nearby	1
	Different	0
Percentage of resurfacing of the defect	100%	3
	66–100%	2
	33–66%	1
	0–33%	0
Percentage of filling of the lesion	100%	3
	66–100%	2
	33–66%	1
	0–33%	0
Regularity of surface tissue	Smooth	2
	Rough	1
	Disorganized	0
Aspect of the junction between the repaired tissue and the adjacent cartilage	Junction in same level	2
	Cracks or overfilling in periphery	1
	Central depression or bulging	0

*The score varied between 0 for normal cartilage and 12 for the lack of repair.

MACROSCOPIC ANALYSIS

All patellae were observed on a binocular magnifying glass (×2) in order to score the repair tissues according to five parameters taking into account the quality of the filling tissue¹⁷. This score varied between 0 and 12, 0 corresponding to the absence of repair and 12 to normal cartilage [Table I].

HISTOLOGICAL ANALYSIS

Fixed patellae were decalcified 1 h with Rapid Decalcifier RDO (Apex engineering products corporation, Canada), dehydrated and embedded in paraffin. Sections of 5 µm were cut in the transversal plane with a microtome, deparaffinized in toluene, and hydrated in a graded serie of ethanol. The sections performed on spontaneous repair tissue and normal cartilage (control group) were stained with hematoxylin-eosin (HES) to evaluate cellularity and morphologic aspect, with toluidine blue (TB) to evaluate proteoglycan content, and picosirius red (SR) to evaluate collagen content. To examine the network organization in both groups, the SR sections were analyzed in polarized light microscopy. For each time point, the quality of the repair tissue was determined according to 11 histological parameters aimed at appreciating morphological and structural organizations, cellular aspect, and distribution of matrix constituents in the repair tissue¹⁴ [Table II]. This score varied between 0 and 28, 0 corresponding to the absence of repair and 28 to a hyaline aspect of the repair tissue.

STATISTICAL ANALYSIS

The differences between global, superficial and deep T2 values were analyzed by an ANOVA followed by a Fisher's t test and compared with control groups with a level of significance $\alpha=0.05$. Concerning chondral repairs (three types of repairs per groups: 'total repair', 'partial repair' and 'hypertrophic repair'), only groups made up by at least four patellae with an identical macroscopic pattern were considered for statistical evaluation.

Results

MACROSCOPY

At D20, repairs have been classified in two categories: 'total repair' (16.7%) and 'partial repair' (83.3%) [Table III]. The 'total repair' zone appeared as a translucent tissue associated with an irregular aspect of its surface, clearly individualized from adjacent normal cartilage. By contrast, 'partial' repairs presented a central depression due to a loss of filling associated with lower macroscopic scores in comparison with full repairing. At D40, the percentage of 'total' repair increased to 75%, but in contrast macroscopic score was not better for 'total repair' and 'partial repair' tissues when compared with D20 [Table III]. At D60, repairs have been distributed in three categories: 'total repair' (50%), 'partial repair' (25%) and 'hypertrophic repair' (25%) [Table III]. In 'total' repairs, we noted similar macroscopic scores when compared with D20 and D40 with a repaired tissue presenting a hyaline cartilage pattern with a smooth or occasionally rough aspect of its surface. Typically, low scores characterized 'partial repair' with a central depression, and 'hypertrophic repair' tissues, which showed a disorganized surface with depression and bulging.

HISTOLOGY

At D20, 'total repair' (16.7%) cartilages presented an irregular surface with a junction on the same level as the adjacent cartilage while the rebuilding of the subchondral bone was not observed in the deep part [Fig. 3 (a)]. The repair tissue (83.3%) was characterized by a fibrotic pattern: fibroblastic cells [Fig. 3 (b)], extracellular matrix weakly staining in PGs [Fig. 3 (c)], strongly marked by picosirius red (collagen) with a globally disorganized network in polarized light microscopy [Fig. 3 (d)]. 'Partial repair' tissue was characterized by an irregular surface located under the level of the adjacent cartilage. Cell morphology and extracellular matrix characteristics were similar to 'total repair' group.

At D40, 'total' and 'partial' repair tissue presented a fibrotic pattern with a higher proportion of chondrocyte type

Table II
Histological score used for histological analysis of the repair tissue according to 11 criteria

Staining	Parameters	Observations	Score*	
HES	Cellular morphology (chondrocyte or fibroblasts)	100% of chondrocytes	4	
		66–100% of chondrocytes	3	
		33–66% of chondrocytes	2	
		0–33% of chondrocytes	1	
		100% of fibroblasts	0	
	Subchondral bone rebuild	Normal	3	
		Limited	2	
		Low	1	
	Percentage of filling	Any	0	
		100%	3	
		50–70%	2	
		25–50%	1	
	Surface regularity	0%	0	
		Smooth or regular	2	
		Slightly irregular	1	
	Interface with the normal cartilage	Irregular or fibrillar	0	
		Junction with two lateral sides	2	
Junction with one side only		1		
Any junction		0		
Vascularity in repair tissue	Absence	1		
	Presence	0		
TB	Percentage of matrix staining by toluidine blue	100	4	
		66–100%	3	
		33–66%	2	
		0–33%	1	
		No stained	0	
SR	Percentage of matrix staining by Sirius red staining	Normal	3	
		0–33% in addition	2	
		33–66% in addition	1	
		66–100% in addition	0	
	Polarized light microscopy to appreciate the collagen network organization in cartilage layers	Superficial zone	Normal architecture	2
			Partially disorganized	1
			Totally disorganized	0
		Transitional zone	Normal architecture	2
			Partially disorganized	1
			Totally disorganized	0
	Radial zone	Normal architecture	2	
		Partially disorganized	1	
		Totally disorganized	0	

*With this score we examined the cell morphology, the matrix content, the organization of the repair tissue and the aspect of the repair tissue compared with the adjacent structures (HES, hematoxyline, eosin, safran; TB toluidine blue, SR Sirius red).

Table III
Macroscopic and histological score of the repair tissue at D20, D40 and D60, type and proportion of repair tissue

Time point	% of total repair	Macro score (/12)	Micro score (/28)	% of partial repair	Macro score (/12)	Micro score (/28)	% of hypertrophic repair	Macro score (/12)	Micro score (/28)
D20	16.7% (N=1)	11±3.4	14	83.3% (N=5)	5.4±3.87	12.8±3.7	–	–	–
D40	75% (N=6)	10.6±1.9	19.7±2.6	25% (N=2)	5.5±0.7	14±1.4	–	–	–
D60	50% (N=4)	9.75±2.06	19.25±1.7	25% (N=2)	7.5±0.7	13±2.8	25% (N=2)	7.5±0.7	12.5±3.5

cells [Fig. 3(a) & (b)]. We observed a partial organization of the superficial part of the repair tissue whereas the deep region was still disorganized [Fig. 3(d)]. The remodeling of the subchondral bone was still not observed in the deep part.

At D60, 'total repair' (50%) were characterized by chondrocytic type cells [Fig. 3(a) & (b)] and an extracellular matrix rich in collagen fibers and proteoglycans [Fig. 3(c)]. Concerning the collagen network we noted a

superficial organization of the network while the deep zone remained still disorganized [Fig. 3(d)]. 'Partial repairs' (25%) had the same characteristics with a lack of filling and organization. 'Hypertrophic repair' tissues (25%), were characterized by a fibrotic pattern: fibroblastic cells [Fig. 3(a) & (b)], extracellular matrix weakly staining in PGs [Fig. 3(c)], strongly marked by picosirius red (collagen), globally disorganized in polarized light [Fig. 3(d)] with depression and bulging. The subchondral bone appeared

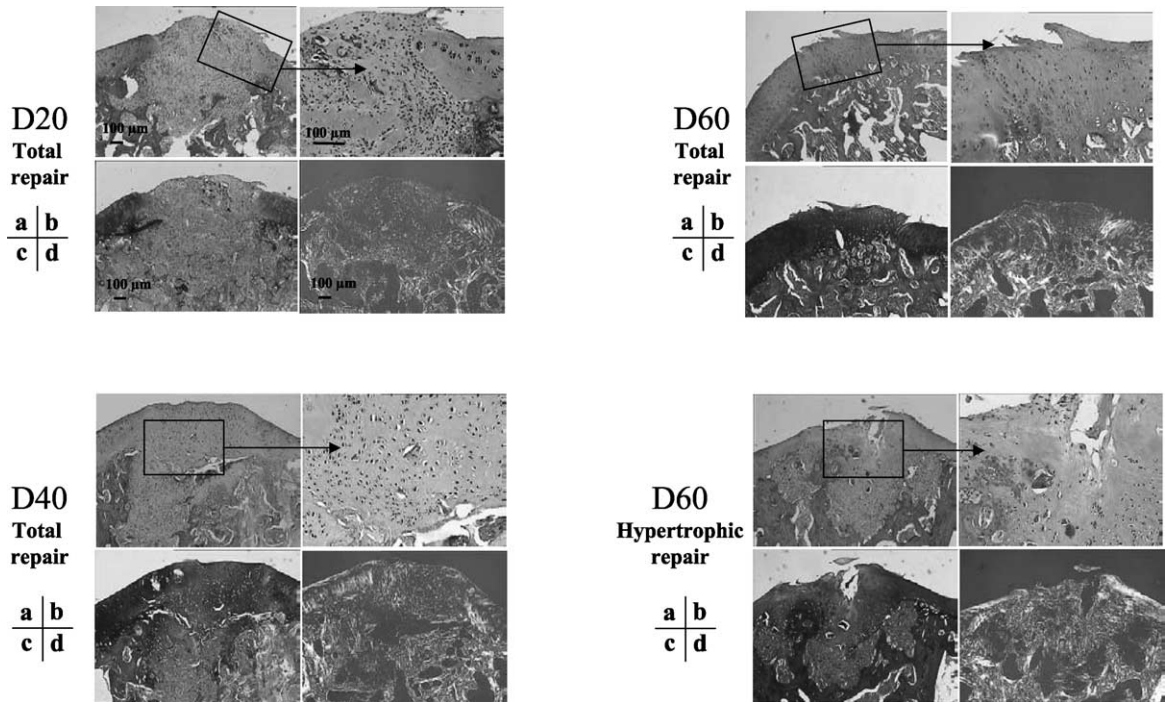


Fig. 3. Histological studies of repaired areas at D20, D40, D60. At D20, the 'total' repair tissue presented an irregular surface (a: HES), fibroblastic cells (b: HES), a weak staining of proteoglycans (c: toluidine blue), and a disorganized network in polarized light microscopy (d: Sirius red in polarized light microscopy). At D40, the 'total' repair tissue was characterized by a higher proportion of chondrocytes (a–b) and a partial organization of collagen fibers in surface whereas the deep region was disorganized (d). At D60, the 'total' repair tissue showed chondrocyte type cells (a–b), an extracellular matrix rich in proteoglycans (c) and organization of collagen fibers in superficial zone (d). The 'hypertrophic' repair tissue was disorganized (d), the type of cells was fibroblastic (a–b) and the proteoglycan content was weak (b).

progressively into the margins of the defect without reaching its central part.

T2 MAPPING OF CONTROL PATELLAE

Morphological analysis

T2 mapping permitted to distinguish each structure of normal patellae: articular cartilage, subchondral bone and trabecular bone. Patellar cartilage appeared as a thin and smooth tissue whose thickness varied between 150 and 250 µm. It appeared in blue-green scale, recovering subchondral bone, typically in black. The trabecular bone is clearly identified as a blue green scale beneath the subchondral bone [Fig. 2].

Quantitative analysis

We noted a progressive and significant decrease of the T2 global values from 7.57 ± 0.35 ms to 6.48 ± 0.20 ms ($P < 0.05$) between D20 and D60, related to the maturation process ($N=8$ per time-point) [Fig. 4 (a)]. A zonal variation of T2 values between chondral superficial zone (high T2 values) and chondral deep zone (lower T2 values) was present whatever the age-group considered ($P < 0.001$) [Fig. 4 (b)].

T2 MAPPING OF SPONTANEOUS REPAIR GROUP

Morphological analysis

Considering the repair zone, we noted a good visual concordance between the macroscopic aspect and the superficial morphological aspect on T2 mapping, whatever the stage considered. Typically, three types of repair (total, partial and hypertrophic) could be macroscopically distinguished [Fig. 1].

Furthermore, T2 mapping allowed to obtain morphological information about the internal structure of the repair tissue and to study the reconstruction of the bone-repair tissue interface not depicted during macroscopic analysis. In our experimental model, osseous modifications were still observed at D60, with an interface between subchondral bone and repair tissue appearing at the margins of the defect.

Quantitative analysis

T2 mapping was realized on focal defects at D20, D40 and D60 on the three types of repair processes. 'Total repair' batch. At D20, T2 global value ($N=1$, 7.14 ms) was similar to controls ($N=8$) [Table IV]. Additionally, no variation of T2 values was observed with cartilage depth. At D40, T2 global values ($N=6$, 9.11 ± 0.40 ms) were higher vs control ($P < 0.05$) [Table IV] and no zonal variation of T2 values was

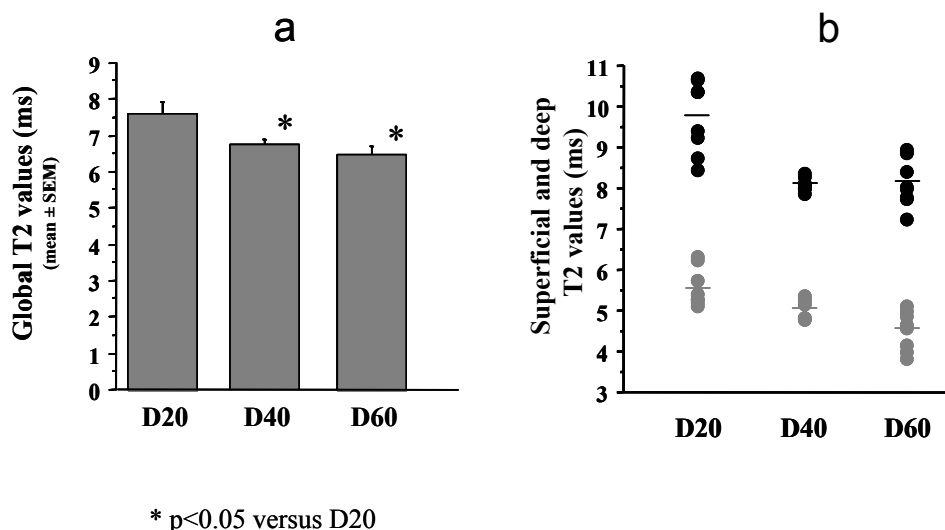


Fig. 4. Global, superficial and deep T2 values of control cartilage at D20, D40 and D60. The global T2 values decreased from 7.6 to 6.4 ms during the maturation process ($P < 0.05$). The zonal variation appreciated as the difference between superficial T2 and deep T2 values was present whatever the age considered ($P < 0.001$).

Table IV
Global T2 values of repair tissue at D20, D40 and D60

Time point	T2 global values (ms) (Mean±S.E.M.)			
	Control	Total*	Partial†	Hypertrophic‡
D20	7.573±0.353 (N=8)	7.146 (N=1)	6.192±1.120 (N=5)	—
D40	6.735±0.166 (N=8)	9.115±0.406 (N=6)	6.479±0.497 (N=2)	—
D60	6.484±0.200 (N=8)	7.311±0.270 (N=4)	5.720±1.378 (N=2)	8.983±1.236 (N=2)

*Total repair tissue presented the same global T2 value at D20 and D60 than controls. In contrast, at D40 the global T2 was significantly higher than controls.

†Partial repair tissue was characterized by low global T2 values.

‡Hypertrophic repair tissue was characterized by significant higher T2 values when compared with the control group ($P < 0.05$).

observed within cartilage depth. When compared with D40, T2 global values in 'total repair' ($n=4$, 7.31 ± 0.27 ms) decreased at D60 and without significant difference vs controls ($N=8$, 6.48 ± 0.20 ms) [Table IV]. At this stage, a zonal variation of T2 values with cartilage depth ($P < 0.05$) was observed, but in a lesser extent vs control group [Fig. 5]. Thus, the magnitude between superficial and deep T2 values was higher in controls (mean of superficial T2 values: 8.140 ± 0.584 ms, mean of deep T2 values: 4.530 ± 0.478 ms) than in total repair rats (mean of superficial T2 values: 9.020 ± 0.846 ms, mean of deep T2 values: 6.192 ± 1.080 ms). In 'partial repair' group, T2 global values were lower than controls throughout the experiment, significant on D20 ($N=5$). 'Partial repair' patellae were also characterized by low superficial T2 values ($P < 0.05$) vs control due to a rough pattern of their surface. No zonal variation of T2 values was noted throughout the experiment. 'Hypertrophic repair' batch ($N=2$) presented a 39% increase of their T2 global values when compared with controls (8.98 ± 1.24 ms vs 6.48 ± 0.20 ms) [Table IV] without any gradient of T2 values varying with depth [Fig. 5].

Discussion

Rat is the archetypal experimental animal for physiological and pharmacological assessments of degenerative or inflammatory articular diseases (osteoarthritis, arthritis) as well as chondral lesions. We have thus developed a model of rat patellar cartilage defect, as an anatomical structure for which T2 mapping, biochemical and histological data have previously been validated^{16,18}. The relevance of this model was based on the localization of this cartilage defect, patellar defects being the most frequent localization during knee OA (88%)¹⁹, and on the fact that surgical coverage of this type of chondral defect, mosaicplasty¹ or chondrocytes graft² might be proposed at early stages before total knee replacement. Numerous studies have studied the clinical relevance of studying focal cartilage lesion models in rabbit²⁰, goat²¹, dog²² and more recently rat^{17,23,24}. With this in mind, several factors must be taken into account: the localization, the depth and the diameter of chondral lesion, the animal species considered and, the influence of rest or cast after the surgery. To the

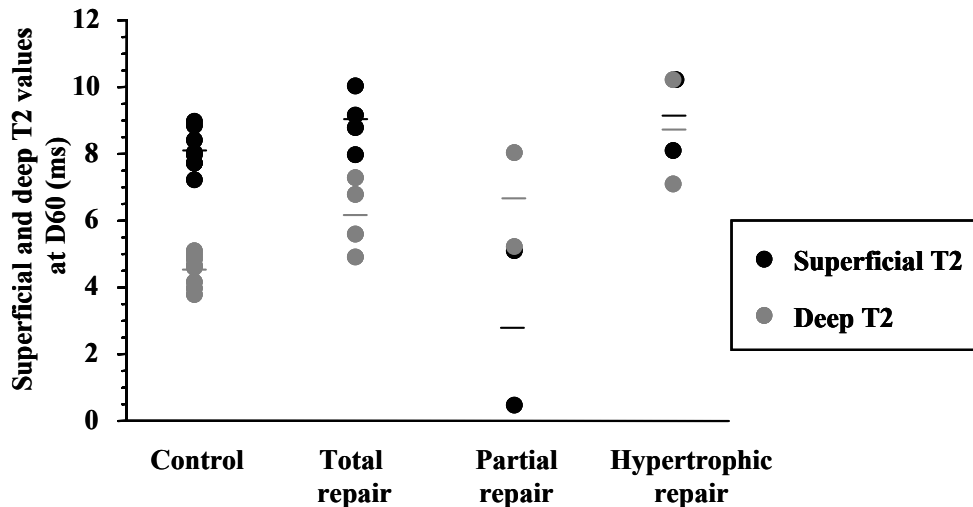


Fig. 5. Superficial and deep T2 values of repair tissue at D60. A zonal variation of T2 in the tissue repair appeared at 60 days in the 'total' repair tissue ($P < 0.05$). The 'partial' and 'hypertrophic' repair tissues did not present any zonal variation of T2 ($P > 0.05$).

best of our knowledge, this pilot study is the first MR study with T2 mapping of an experimental model of patellar focal lesion in the rat with macroscopical and histological confrontations.

Concerning rat studies, in a transversal cartilage defect performed on trochlear cartilage, Yoshioka *et al.*²⁰ demonstrated a partial filling of the defect after 3 weeks followed by a total fibrotic tissue presenting a rough surface, with cells randomly distributed. In the same study, during a longitudinal defect model, the scarred tissue was filled by a white tissue at 3 weeks which presented a hyaline pattern on macroscopic and histological analysis at 8 weeks without 'hypertrophic' or 'partial' repairs. In the focal patellar model presented here, the profile of scarring tissue in 'total repair' batch exerted intermediate histological characteristics when compared with those described by Yoshioka *et al.*²³. On the other hand, we noted 25% of 'hypertrophic' tissue at D60 probably due to the patellar localization and the type of the defect. This latter hypothesis concerning the tissue is based on clinical findings related to patellar grafts where hypertrophic tissue was observed in 23% of Brittberg's chondrocyte grafts² and in 25% during mosaicplasty technique¹. Additionally, in the model presented here, rats were free-moving after surgery since Espanha *et al.*²⁴ demonstrated that free motion after surgery in rats did not affect the quality of the repair tissue and could increase PG content as demonstrated by histological study.

MR clinical studies performed on T2-weighted sequences permitted to appreciate the morphological evolution of grafts and to differentiate normal cartilage from delamination or hypertrophic tissues^{6,25}. Moreover, when compared with arthroscopic examination, MRI is able to assess the internal structure of repair tissue, to follow its evolution, and to evaluate the cartilage-bone interface. Thus, Alparslan *et al.*⁶ showed that normal graft presented initially an internal structure in hypersignal intensity before becoming normal one-year later on T2-weighted sequences. Conversely, the hypertrophic and delaminated grafts remained in hypersignal whatever the time studied. In all cases, the trabecular bone surrounding the defect presented an edema in high signal intensity present at 12 months, but disappearing at 24 months.

Although our preliminary current results seem promising, neither published studies permitted to establish quantitatively any comparison between MRI and histological data. A quantitative approach has been recently performed by Gillis *et al.*⁸, who determined the ability of T1 mapping technique plus contrast agents to assess the proteoglycan content in 10 autologous chondrocytes grafts at 2, 6, 12, 18, and 24 months. Before 6 months the proteoglycan content was weak, progressively increasing to 92% at 12 months and then remaining stable at 24 months. Nevertheless, this study did not evaluate the organization of the collagen network such as recently demonstrated by using T2 mapping technique²⁶.

Our study also confirmed the ability of T2 mapping to identify several morphological patterns previously described in the clinics: 'total', 'partial', and 'hypertrophic' repair tissues^{6,25}. When compared with clinical studies, delamination pattern was not observed on T2 mapping as well as on macroscopic and histological data. This particular point may be related to the fact that, in the present work, the defect is only filled by a blood clot issued from the subchondral bone, and not by a graft (mosaicplasty or autologous chondrocytes grafts). In all cases of repair tissue, a tissue characterized by high T2 values replaced the subchondral and the trabecular bone.

We also confirmed in the control group, a progressive but significant decrease of T2 global values from 7.6 to 6.4 ms between D20 and D60. In a previous paper¹², we showed that this decrease, related to the maturation process, was highly correlated to the variations of the matrix content (collagen ($r = -0.73$) and proteoglycan ($r = 0.68$)). 'Total repair' tissue was characterized by high global T2 values vs control group at D40 whereas this difference disappeared at D60. This increase at D40 is probably inherent to a chondral edema related to the low content of proteoglycan highlighted on histology. In fact, we [Watrin *et al.*, submitted] and others²⁷ have previously demonstrated that a depletion of PGs led to an increase of free space inside the matrix that will be filled by water. An ancillary higher mobility of water protons may thus be responsible for the increase of T2 values. In contrast, at D60, 'total repair' T2 global values were similar to those of control group, likely due to the

hyaline aspect of repair tissue as depicted histologically. The 'hypertrophic tissue', only depicted at D60, was characterized by high T2 global values potentially corresponding to a disorganized fibrocartilage, poorly marked by PG staining. This low content of PGs, responsible for the excess of proton mobility, may lead to inherent high global T2 values.

Our study showed that the variations of T2 global values in the repair tissue in rat were similar to those reported by Alparslan *et al.*⁶ in terms of signal intensity variation of the grafted tissue on T2-weighted sequence. The hypertrophic or edematous grafts are characterized by high signal intensity in T2 weighted sequences and high T2 global values on T2 mapping while 'total repair' tissue does not present any significant difference in terms of signal intensity or T2 global values when compared with normal cartilage. The zonal variation of T2 values (high T2 values in surface and low T2 values near the calcified cartilage) is classically observed in human cartilage as well as in numerous animal species^{9,11,12,28}. Recently, Nieminen *et al.*²⁶ demonstrated that the zonal variation of T2 values was correlated ($r=0.91$) with the collagen network organization on bovine osteochondral plugs. In our study, the zonal variation of T2 values was present in normal rat patellar cartilage throughout the study. This variation was not depicted in repair tissue where the collagen network was not organized, e.g. in 'hypertrophic' and 'total' repair tissue at D20 and D40. Conversely, at D60, this zonal variation was observed on T2 mapping, when the repair tissue presented a collagen network organization in the superficial zone on polarized light microscopy, thus supporting its importance in determining this zonal gradient.

The simple qualitative analysis of T2 map permits to differentiate normal from abnormal patterns of repair tissues (hypertrophic and partial repairs). However, the quality of the repair tissue in terms of matrix content and network organization required T2 quantitative tools such as global T2 values and zonal T2 variations. This last parameter is more relevant to characterize the hyaline cartilage organization. The interest of this experimental approach is to compare T2 map results to histological analysis, studies which can not be performed on clinical practice. This promising technique actually available on clinical imagers¹¹ offers accurate methods to assess the repair tissue process in chondral defect. However, the assessment of lesions occurring in OA disease required a different experimental approach as well as new and adapted quantitative tools. The inherent limitations of this non invasive technique depends on the classical factors related to MRI (cost, access, claustrophobia . . .) and the duration of the acquisition (30 min on a 1.5 T imager) which would be reduced with improved technology.

Conclusion

The results presented here permit to characterize macroscopically and microscopically the patterns of a focal chondral lesion in rat patella and to define several types of repair patterns ('total', 'partial' and 'hypertrophic') over 60 days. We demonstrate herein the potentiality of T2 mapping to evaluate their tissular characterization on an 8.5 T imager. MR T2 map may be performed on a focal region of interest and permits to obtain qualitative and quantitative parameters concerning the graft as well as the adjacent tissue (cartilage and subchondral bone). We confirm the ability of T2 mapping to differentiate macroscopically 'total'

from 'partial' or 'hypertrophic' repair patterns, classically depicted on T2 weighted sequence used in clinical practice. Furthermore, T2 mapping permits to obtain two informative quantitative parameters: T2 global values in assessing biochemical matrix content and T2 zonal variation in evaluating the integrity of the collagen network organization. Finally, T2 mapping could offer a 'MR biopsy' liable to evaluate, qualitatively and quantitatively, *in vivo* cartilage engineering. Beyond experimental studies, this non invasive tool currently developed on clinical imagers could be used to evaluate qualitatively and quantitatively human cartilage graft.

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