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Research Article

High-field MRI exploration of the structural effects of cellular matrix™ on articular cartilage in knee osteoarthritis: A pilot study in 6 patients

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Objective: To analyze the potential modulatory effect of Cellular Matrix, a new medical device de-signed for the one-step preparation of platelet-rich plasma in presence of hyaluronic acid, on the structure of articular cartilage in patients suffering from knee osteoarthritis using high-field Magnetic Resonance Imaging measurements of longitudinal relaxation time after gadolinium injection.

Methods: The treatment consisted of a series of 3 intra-articular injections scheduled at D0, D60 and D180 into the affected knee of six patients with Kellgren-Lawrence grades of 1.5 to 3. Magnetic Resonance Imaging acquisitions were performed before the first injection at D0 (baseline), at D180 (just after the third injection) and at D270 (3 months after the third injection). The efficacy criterion was the variation of T1 relaxation time in different selected cartilage regions.

Results: Our study reveals a positive" time-dependent" structural effect of the combination of PRP and HA obtained with Cellular Matrix on the proteoglycan content of the knee joint cartilage. At D180, the weight-bearing areas were involved in two patients with Kellgren-Lawrence grades equal to or greater than 2. At D270, 5 patients showed an initial improvement in the weight-bearing area; only one patient with early external femoropatellar osteoarthritis (with a Kellgren-Lawrence grade of 1.5) had no improvement.

Conclusion: This pilot study demonstrates for the first time the modulatory effect on the structure of the knee joint cartilage of a combination of platelet-rich plasma and hyaluronic acid prepared with a specially dedicated medical device (Cellular Matrix) during the course treatment. Cellular Matrix could therefore be considered a Disease Modifying Osteoarthritis Device.

Keywords: cellular matrix • osteoarthritis • pilot

Introduction

While osteoarthritis (OA) is the most common cause of pain and disability among people over 50 years of age [1], knee OA is becoming a real public health issue as populations age. Knee OA is an underestimated condition. Its increasing prevalence [2-4] has been causally linked with obesity [5]. In the United States, surgeons performed 686,000 knee replacements in 2009, and projections predict the implantation of 1,520,000 prostheses in 2020 and 3,480,000 in 2030. Prosthetic revision rate (unicompartmental or total) continues to progress. A 600% increase is expected by 2030 [6].

Intra-articular injection of Hyaluronic Acid (HA), referred to as viscosupplementation, represents a recognized treatment for knee OA. Many clinical trials testing different HA preparations have been performed in humans, some of which report results versus saline placebo. Most of these studies conclude that HA is superior to a saline placebo, whatever its molecular weight [7-13].

More recently, Platelet-Rich Plasma (PRP) injections have proven to be an interesting treatment option [14-23]. The potential efficacy of PRP in the treatment of cartilage lesions has already been evaluated *in vitro*; particularly, PRP has been shown to increase the synthesis of proteoglycans and collagen in the extracellular matrix of cultured intervertebral disc cells [24]. However, very few studies have documented a possible modulatory effect of PRP on cartilage structure in Humans to date.

In recent years, it has become more and more obvious that the association of PRP with HA could provide added benefit for the treatment of joint degenerative diseases, due to their different mechanisms of actions to modulate the disease process [25-32].

Joint cartilage is made of water (60%-80%) and chondrocytes surrounded by an extracellular matrix [33]. This matrix is composed of type II collagen (5%-10%) and proteoglycans (10%-20%) (PG) [34]. Cartilage damage in osteoarthritis is accompanied by biochemical changes in the collagen network and proteoglycans. The loss of proteoglycans has been associated with the early phases of osteoarthritis based on studies conducted in animal models [35,36] and anatomical parts [37,38]. These biochemical alterations, which escape conventional radiology techniques, can be detected by Magnetic Resonance Imaging (MRI), which represents therefore a tool of choice as a non-invasive approach to osteoarthritis.

Different functional approaches by MRI based essentially on relaxation time measurements coupled or not with the injection of a contrast agent have been developed. The T1 relaxation time measurement after injection of a gadolinium (Gd)-based contrast agent is the most commonly used technique [39] with measurements made about 90 minutes after the injection phase. Delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) is based on the demonstration that Gd distributes in inverse relationship to cartilage PG content, leading to a reduction of T1 relaxation time.

Van Tiel described and used a promising reproducible methodology based on this technique to explore the potentially structural effect of HA in early stage knee OA, unsuccessfully [40].

Our study aimed at demonstrating with the same validated methodology that PRP combined with HA can be structurally effective on articular cartilage in knee OA. Using an innovative medical device allowing the preparation of autologous PRP in presence of HA in a one-step procedure and in close circuit (Cellular Matrix™), this collaborative work between rheumatologists and the Centre National de Recherche Scientifique (CNRS) has made it possible to study the effect of a combination of PRP and HA on the cartilage of the knee using high-field 3 Tesla (3T) MRI measurements. The safety and efficacy of Cellular Matrix has already

been assessed in several clinical studies, including a recent one still showing a clinical benefit on pain and function 4 years after a 3-injection course treatment [41].

Objectives of the study

To analyze the potential modulatory effect of the combination of PRP and HA prepared with Cellular Matrix (CM-PRP-HA) on the structure of the articular cartilage in patients suffering from knee OA using high-field MRI measurements of longitudinal relaxation time after gadolinium injection (dGEMRIC), a scientifically recognized indirect index of proteoglycan (PG) content.

Patients and methods

Patients

Six patients were included after they provided their written informed consent. Inclusion criteria were as follows: participants older than 18 years, knee pain duration longer than 3 months and radiographic knee OA with Kellgren-Lawrence (KL) grades of 1 to 3 [24,42]. Exclusion criteria were: contraindications to MRI, renal insufficiency (glomerular filtration rate<60 ml/min), knee surgery within the last year, recent viscosupplementation or glucocorticoid injection. The study protocol was authorized by the French National Authority for Health (ANSM) and approved by local Ethics Committee (CPP Sud-Est I). The study was conducted according to Good Clinical Practice and guidelines of the Declaration of Helsinki.

Treatment

The combination of PRP and HA was obtained using the Cellular Matrix device, as per instructions for use supplied with the kit. Cellular Matrix, manufactured by Regen Lab SA, Le Mont-sur-Lausanne, Switzerland, is a class III medical device. It allows for the extemporaneous preparation of a combination of autologous PRP and non-crosslinked HA gel 2% (CM-PRP-HA) intended to be used for intra-articular injection (Figure 1). The HA used (2 ml) has a molecular weight of 1550 kDa. Each patient received a series of three intra-articular injections of CM-PRP-HA at D0 (baseline), D60 and D180, as described by Renevier et al. [41].

MRI acquisition

MRI acquisitions were performed before the injection of CM-PRP-HA at D0 (baseline), D180 (just after the third injection) and D270 (3 months after the third injection). Before each

Figure 1. One step procedure for the preparation of a combination of PRP and HA with cellular matrix device.

Following blood withdrawal, the tube is centrifuged for 5 minutes at 1500 g. During this centrifugation, the gel migrates to form a barrier that mechanically separates the plasma and platelets from the other blood components (red and white blood cells). This supernatant containing plasma and concentrated platelets is PRP. The HA gel, which was located at the bottom of the tube before centrifugation, migrates to the top of the tube and sits above the PRP fraction. This allows for an easy mixing of HA and PRP after centrifugation by gentle and repetitive inversions and rotations of the tube. The final PRP/HA combination is collected using a blood transfer device and is ready to be used by the physician. The full procedure is done is close-circuit.

MRI session, a double dose (0.2 mmol/kg) of gadoteric acid (Dotarem®, Guerbet, France) was injected intravenously approximately 95 min before the MRI session. Patients were then asked to exercise for 15 minutes on a cyclo-ergometer at a comfortable rate and pedaling frequency in order to promote the contrast agent distribution within the knee articular cartilage as previously described [39,40]. MRI measurements were started after an additional 80 min resting period. MR imaging was performed on a 3.0 Tesla MRI scanner (Verio, Siemens Germany) using a set of phase array surface coils positioned above and below the knee (Figure 2). After a localization procedure using scout images, quantitative sagittal T1 mapping was performed using a dual-flip angle 3D GRE sequence with the following parameters as previously described [39,40,43] flip angles: 6 and 33°, TR: 15 ms, TE: 2.58 ms, Field-of-view (FOV): 144 mm; slice thickness: 3 mm, slice oversampling: 28.6%, matrix size: 384 * 384, bandwidth: 380 Hz/pixel, in plane resolution 0.4 * 0.4 mm. The resulting scan time was 4.5 min for a slab with 28 slices. In addition to the quantitative map, morphological evaluation was performed using a sagittal T1-W turbo spin echo sequence (TR-TE: 700-18 ms,
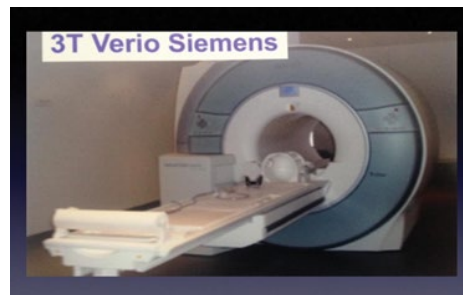
Figure 2. 3.0 tesla MRI scanner used at the Center for metabolic exploration by magnetic resonance (CEMEREM, Marseille).
MRI : Magnetic Resonance Imaging; CEMEREM: Centre d'exploration métabolique par résonance magnétique
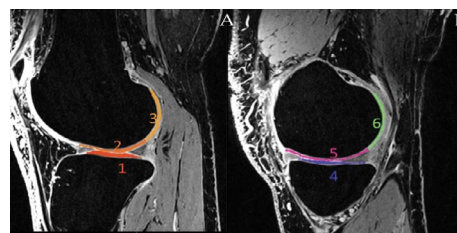
Figure 3. Six masks manually segmented. 1 & 2 and 4 & 5: Weight-bearing anterofemoral (2 & 5) and tibial (1 & 4) areas, lateral (A) and medial (B); 3 & 6: Non-weight-bearing posterofemoral areas, lateral (A) and medial (B).

voxel size: 0.5 * 0.4 mm), and both sagittal and coronal proton-density turbo spin echo sequences including a fat saturation scheme (TR-TE: 4000-37 ms, voxel size 0.5 * 0.4 mm and TR-TE: 3800-37 ms, voxel size: 0.4 * 0.4 mm). The FOV was 130 mm. the total scan time for the morphological evaluation was 7.1 min.

Data analysis

From the T1W-MRI baseline dataset, a central slice was selected in the external and internal tibiofemoral areas. For each area, three cartilage regions of interest (masks) were manually drawn by an expert surgeon using FSL View, the 3D viewer included in the FSL toolbox [39,40]. These 3 regions consisted of the weight-bearing cartilage of the femoral condyles (Areas #2 and #5), the posterior non-weight-bearing cartilage of the femoral condyles (Areas #3 and #6) and the weight-bearing cartilage of the tibial plateaus (Areas #1 and #4) (Figure 3). Using a non-linear registration method, these manual masks were propagated to the superior and inferior slices and then to the MRI datasets recorded at D180 and D270. For this purpose, T1W-MRI obtained at D180 and D270 have been registered into the baseline T1W-MRI dataset. The corresponding T1 maps have also been resampled using the

baseline T1W-MRI dataset as a target.

All registration and resampling have been performed using the ANTS library (http://stnava.github.io/ANTs/) tools [44]. This registration process eliminated subjective visual slice matching and additional manual segmentations. As previously reported, cartilage regions with long T1 relaxation time have relatively high glycosaminoglycan (GAG) content compared to cartilage regions with short T1 relaxation time which indicates reduced GAG content (refs). In order to avoid all possible partial volume effects from the cortical bone, a very conservative segmentation procedure was used so that cartilage pixels in the close vicinity of bone pixels were systematically excluded. It has been previously suggested that a 95 ms difference in T1 relaxation time corresponding to a 19% change could be considered as clinically relevant and indicative of an improved cartilage GAG content as measured by 3D dGEMRIC at 3.0 Tesla [39]. We used a similar approach in order to compare the post-contrast T1 values at different times.

Results

Radiological and WORMS score

The radiological scores determined by two clinicians in charge of recruiting patients on the Kellgren-Lawrence (KL) scale (42) was between 1.5 and 3 (Table 1). The WORMS score (Figure 4) for cartilage ranged from 0 (patient, P1) to 32.5 for the patient 6 (P6) (Table 2) [45]. As shown in Figure 5, both scores were highly correlated (R2=0.8).
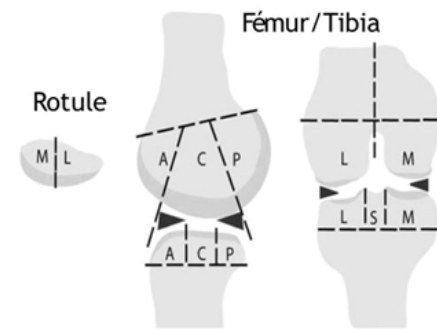
Quantitative MRI analyses

Analysis #1: D0 *vs* D180

External compartment: At D0, the mean T1 values (± SD) were 358 ± 109, 373± 82 et 415 ± 123 for the tibial, anterofemoral and posterofemoral zones, respectively (Table 3 and Figure 6). The

Figure 4. WORMS: Whole organ magnetic resonance imaging score.

At time D0, the WORMS score was established for each patient according to the following items. Cartilage (signal and morphology), Sub-articular compartment (bone marrow, cysts, osteophytes, bone wear), meniscus (integrity) cruciate ligaments (integrity). Analysis area: medial, lateral, tibial spine, anterior, posterior, central.
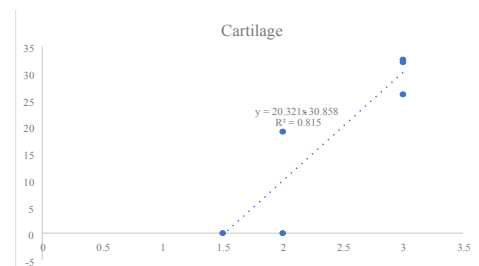
Figure 5. Correlation between Kellgren-Lawrence grade (x-axis) and WORMS (y-axis).

Table 2. WORMS scores of the patients included in the study.

Score WORMS Cartilage	Score		rank
	WORMS Total	rank	
P1	0	1	6	6
P2	32.5	209	1	1
P3	21	62	5	5
P4	19	75	4	3
P5	26	85	3	2
P6	32	64	2	4

coefficients of variation (CV) were comparable between zones (22 to 31%). As shown in Table 3, the T1 values measured at D180 were not different from those measured at D0.

Internal compartment: At D0, the mean T1 values (± SD) were 284 ± 63, 293± 41 et 387 ± 45 respectively for the tibial, anterofemoral and posterofemoral zones, respectively (Table 4 and Figure 7). The coefficients of variation (CV) were comparable between zones (12 to 22%). As shown in Table 4, the T1 values measured at D180 were not different from those measured at D0. For comparative purposes, we

Table 1. Radiological scores of the patients included in the study, according to the Kellgren-Lawrence grading system.

	KL score	Type of osteoarthritis
P1	1.5	Early external femoropatellar
P2	3	Global with effusion
P3	2	Internal femorotibial
P4	2	External femorotibial and patellofemoral
P5	3	Internal femorotibial
P6	3	Internal femorotibial

Table 3. External compartment: T1 values measured at D0 and D180.						
Compartiment externe						
	Tibia		Fem ANT		Fem POST	
	J0	J180	J0	J180	J0	J180
P1	340	309	362	332	344	327
P2	214	211	262	240	339	220
P3	489	695	487	717	588	682
P4	436	356	422	263	549	324
P5	250	239	304	253	291	197
P6	418	366	403	472	379	479
moyenne	358	363	373	379	415	371
SD	100	159	75	170	112	166
CV (%)	28	44	20	45	27	45
SD: Standard deviation; CV: Variation coefficient						

Table 4. Internal compartment: T1 values measured at D0 and D18.						
Compartiment interne						
	Tibia		Fem ANT		Fem POST	
	J0	J180	J0	J180	J0	J180
P1	335	194	353	209	421	264
P2	208	160	289	240	358	365
P3	305	286	327	329	389	497
P4	239	154	257	182	361	253
P5	248	278	246	159	335	297
P6	371	265	286	405	458	465
moyenne	284	223	293	254	387	357
SD	57	55	38	86	41	95
CV (%)	20	25	13	34	11	27
SD: Standard deviation; CV: Variation coefficient						
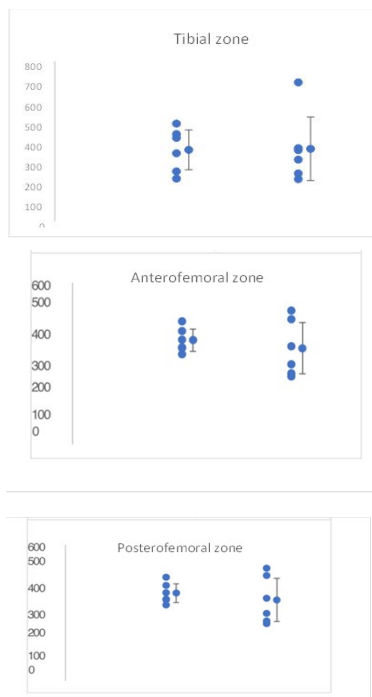
Figure 6. T1 values (ms), as measured before (left-hand side) and after (right-hand side) treatment in the three zones of the external compartment.
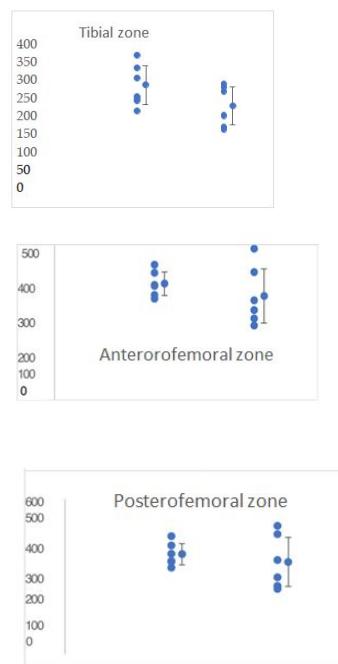
Figure 7. T1 values (ms), as measured before (left-hand side) and after (right-hand side) treatment in the three zones of the internal compartment.

calculated the ratio of T1 values between the external and internal compartments. For the tibial and anterofemoral compartments, the values were generally higher for the external compartment. This difference was not found for the posterofemoral compartment (Table 5).

Zone score D180: Four patients had no improvement in the T1 values, while two patients had localized improvements. More specifically, P3 showed improvements in the tibial and

anterofemoral zones of the external compartment and in the posterior femoral zones of the inner compartment. For P6, the increases were localized at the level of the posterofemoral zone (external compartment) and the anterofemoral zone of the internal compartment (Table 6).

Total score at D180: Table 7 summarizes all this data and presents the total score calculated on this basis. In this context, Patient P3 had the best outcomes with improvements on both the

Table 5. Ratio of T1 values between external and internal compartments.						
C Ext / C int						
	Tibial		Anterofemoral		Posterofemoral	
	D0	D180	D0	D180	D0	D180
P1	1.02	1.6	1.02	1.59	0.82	1.24
P2	1.03	1.32	0.91	1	0.94	0.6
P3	1.6	2.43	1.49	2.18	1.51	1.37
P4	1.83	2.31	1.64	1.45	1.52	1.28
P5	1.01	0.86	1.24	1.59	0.87	0.66
P6	1.13	1.38	1.41	1.17	0.83	1.03
Mean value	1.27	1.65	1.28	1.49	1.08	1.03
SD	0	1	0	0	0	0
CV (%)	28	37	22	27	31	32
C Ext: External compartment; C int: Internal compartment; SD: Standard deviation; CV: Coefficient of variation						

Table 6. Zone score at D180.						
	External compartment			**Internal compartment**		
	Tibial	Antero-femoral	Postero-fermoral	Tibial	Antero- femoral	Postero-femoral
P1	0	0	0	0	0	0
P2	0	0	0	0	0	0
P3	1	1	0	0	0	1
P4	0	0	0	0	0	0
P5	0	0	0	0	0	0
P6	0	0	1	0	1	0

Table 7. Total score at D180.								
	Cpt EXT	**Cpt INT**	**Total**	**Rank**	**Weight-bearing areas**	**Rank**	**Non-weight bearing areas**	**Rank**
P1	0	0	0	3	0	3	0	3
P2	0	0	0	3	0	3	0	3
P3	2	1	3	1	2	1	1	1
P4	0	0	0	3	0	3	0	3
P5	0	0	0	3	0	3	0	3
P6	1	1	2	2	1	2	1	1
Cpt ext: External compartment; Cpt int: Internal compartment								

weight-bearing and non-weight-bearing zones. Patient P6 also showed signs of improvement in both areas.

Analysis #2: D0 *vs* D270

External compartment: At D0, the mean T1 values (± SD) were 358 ± 109, 373 ± 82 et 415 ± 123, respectively for the tibial, anterofemoral and posterofemoral zones, respectively (Table 8). The coefficients of variation (CV) were comparable between zones (22 to 31%). As shown in Table 8, the T1 values measured at D270 were not different from those measured at D0.

Internal compartment: At D0, the mean T1 values (± SD) were 284 ± 63, 293± 41 et 387 ± 45, respectively for the tibial, anterofemoral and posterofemoral zones, respectively (Table 9). The coefficients of variation (CV) were

comparable between zones (12 to 22%). As shown in Table 9, the T1 values measured at D270 were not different from those measured at D0. For comparative purposes, we calculated the ratio of T1 values between the external and internal compartments (Table 10). For the tibial and anterofemoral compartments, the values were generally higher for the external compartment. This difference was not found for the posterofemoral compartment.

Zone score at D270: At this stage and given the small number of values, we opted for an individual analysis strategy by adapting the results of a previous study [40]. We chose 19% as a significant threshold of increase. This threshold was calculated on the basis of the T1 (500 ms) values reported by van Tiel et al and the 95 ms value reported as significant [39, 40]. In other

Table 8. External compartment: T1 values measured at D0 and D270.						
External compartment						
	Tibial		Anterofemoral		Posterofemoral	
	J0	J270	J0	J270	J0	J270
P1	340	346	362	388	344	408
P2	214	288	262	324	339	313
P3	489	637	487	693	588	739
P4	436	295	422	310	549	259
P5	250	440	304	348	291	284
P6	418	441	403	349	379	445
Mean values	358	408	373	402	415	408
SD	100	120	75	133	112	162
CV (%)	28	29	20	33	27	40
SD: Standard deviation; CV: Variation coefficient						

Table 9: Internal compartment: T1 values measured at D0 and D270						
Internal compartment						
	Tibial		Anterofemoral		Posterofemoral	
	J0	J270	J0	J270	J0	J270
P1	335	261	353	302	421	339
P2	208	147	289	217	358	357
P3	305	409	327	450	389	616
P4	239	287	257	287	361	330
P5	248	238	246	299	335	369
P6	371	776	286	247	458	408
Mean values	284	353	293	300	387	403
SD	57	204	38	73	41	98
CV (%)	20	58	13	24	11	24
SD: Standard deviation; CV: Variation coefficient						

Table 10. Ratio of T1 values between external and internal compartments.						
C Ext / C in						
	Tibial		Anterofemoral		Posterofemoral	
	J0	J270	J0	J270	J0	J270
P1	1.02	1.33	1.02	1.28	0.82	1.2
P2	1.03	1.96	0.91	1.49	0.94	0.88
P3	1.6	1.56	1.49	1.54	1.51	1.2
P4	1.83	1.03	1.64	1.08	1.52	0.79
P5	1.01	1.85	1.24	1.16	0.87	0.77
P6	1.13	0.57	1.41	1.41	0.83	1.09
Mean values	1.27	1.38	1.28	1.33	1.08	0.99
SD	0	1	0	0	0	0
CV (%)	28	38	22	14	31	20
SD: Standard deviation; CV: Variation coefficient						

words, a 19% increase in the T1 value was considered as a sign of improvement (score = 1) while an increase of less than 19% was assigned a score of 0. Five patients had localized T1 values improvements. More specifically, P3 showed improvements in all areas (external and internal compartments). For P6, an increase was localized in the tibial zone of the internal compartment. The other three patients P2, P4 and P5 have each shown an initial improvement each time at the weight-bearing areas. On the other hand, patient P1, who only suffered from an early stage of knee osteoarthritis, showed no change. Table 11 summarizes these zone scores.

Total score at D270: Table 12 summarizes all this data and presents the total score calculated on this basis. In this context, patient P3 had the best outcomes with improvements on both the weight-bearing and non-weight-bearing zones. Patient P6 showed signs of improvement only in the weight-bearing area.

	External compartment			Internal compartment		
Table 11. Zone score at D270.						
	Tibial	Antero-femoral	Postero-fermoral	Tibial	Antero- femoral	Postero-femoral
P1	0	0	0	0	0	0
P2	1	1	0	0	0	0
P3	1	1	1	1	1	1
P4	0	0	0	1	0	0
P5	1	0	0	0	1	0
P6	0	0	0	1	0	0
SD: Standard deviation; CV: Variation coefficient						

	Cpt EXT	Cpt INT	Total	Weight-bearing areas	Non-weight- bearing areas
Table 12. Total score at D270.					
P1	0	0	0	0	0
P2	2	0	2	2	0
P3	3	3	6	4	2
P4	0	1	1	1	0
P5	1	1	2	2	0
P6	1	0	1	1	0

Discussion

Our study is based on the well documented inverse relationship between Gadolinium penetration into the cartilage and T1 relaxation time, and the relationship between T1 relaxation time and the proteoglycan content in cartilage [39,40]. In general, the T1 values reported in this study are lower than the values reported in the literature for healthy subjects but also for subjects with osteoarthritis [39,40]. In accordance with the results reported in the literature [43,46-49], this decrease clearly indicates a significant loss of proteoglycans. This loss seems to be less pronounced in the external compartment than in the internal compartment, in line with previous work [39,40].

In addition, T1 values were correlated with WORMS radiological score values, strengthening the adequacy of T1 measurements as a quantitative tool for cartilage monitoring. Such a conclusion has also been proposed on the basis of a comparative analysis between T1 measurements and T1rho measurements [50].

It should be noted that the measurement method we chose for the T1 relaxation time was different from the one used in the work of Van Tiel et al. [39,40]. This could explain the differences in values between the two studies without impacting our comparative analysis. On the basis of this difference, we chose to adapt our analysis method to the 95 ms threshold previously reported [39] for T1 values close to 500 ms. Consequently, we considered a 19% increase in the T1 value as an indication of improvement in the proteoglycan content.

Statistically, (at mean values, Mann Whitney paired series tests were performed with a statistical threshold $p<0.05$), there was no difference between the measurements at D0 and D180, nor between D0 and D270, which probably reflects the small number of subjects. However, based on an individualized analysis and a 19% threshold increase in the T1 value, five patients showed localized improvements.

At D180, these improvements were localized in the tibial and anterofemoral areas of the outer compartment and in the posterofemoral area of the inner compartment for Patient P3. For patient P6, these improvements were localized in the posterofemoral area (outer compartment) and the anterofemoral area of the inner compartment. In both cases, the weight-bearing areas were involved in these two patients who had a Kellgren-Lawrence score equal to or greater than 2.

At D270, we observed a consolidation for Patient P3 with a score that tripled. In this case, all cartilage areas showed improvement. For Patient P6, the score was reduced from 2 to 1 with an improvement in the tibial area of the internal compartment. While Patients P2, P4 and P5 showed an initial improvement in the weight-bearing area, only Patient P1 had no improvement.

Our study therefore reveals a positive" time-dependent" structural effect of the combination of PRP and HA obtained with Cellular Matrix on the proteoglycan content of the knee joint cartilage. Patients responded relatively quickly

given the avascular and paucicellular nature of the cartilage tissue which is characterized by a very slow turn-over in physiological situations and even more so in the hostile inflammatory context of knee osteoarthritis.

Beside growth and regeneration factors, the platelet secretome contains anti-inflammatory cytokines; PRP probably acts through this dual effect [51]. Hyaluronic acid, on the other hand, is expected to act as a support potentiating PRP activity [32] and have a facilitating role that may potentiate or maximize tissue response to growth factors [52].

Clinically, all treated patients experienced improvement in pain and stiffness in accordance with the study of Renevier et al. [41]. This confirms the clinical relevance of the variations in T1 values defined by Van Tiel et al. [39,40] (95 ms for values close to 500 ms or a 19% difference).

Conclusion

This is a pilot, proof of concept study, aiming at demonstrating for the first time the modulatory effect on the structure of knee joint cartilage of a combination of PRP and HA prepared with a specially dedicated medical device (Cellular Matrix).

The small number of patients did not allow for a relevant statistical study; however, the individual analysis strategy adapted from Van Tiel et al. [39,40] was appropriate. Thus, the individual comparative analysis considering a 19% increase in the T1 value as significant clearly indicated that 2 out of 6 patients had positive outcomes at D180, while 5 out of 6 patients had improvements at D270. One patient aggregated positive outcomes over the 6 zones studied. Only Patient 1 did not show any improvement.

A modulatory time-dependent effect on cartilage structure is thus demonstrated. The clinical confrontation is unequivocal with improvement of all treated patients for both pain and stiffness scores, in line with the Renevier's study [41]. In the end, this research work demonstrates that the combination of PRP and HA (Cellular Matrix) is structurally effective at D270. Cellular Matrix can therefore be considered as a true anti-osteoarthritis treatment with a proven structural effect on knee joint cartilage in Humans. A large-scale multicenter European study with the same methodology and with the same parameters but with a long-term MRI analysis scheduled at D360 is therefore required in order to confirm our positive preliminary data.

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