Original Article

Establishing an *in vitro* model of MR-T1ρ imaging technology to quantify nucleus pulposus tissue proteoglycans: a preliminary study

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Background: The aim of the present study was to construct an *in vitro* model of degenerated nucleus pulposus with different combinations of biochemical components, and to find an *in vitro* model for the early degeneration of nucleus pulposus suitable for the detection of magnetic resonance T1rho (MR-T1ρ) sequence for the early diagnosis of degeneration of lumbar intervertebral disc.

Methods: The proteoglycan concentration gradient in the first experimental group was 5%, with a concentration range of 7 samples *in vitro* models from 5% to 35%. The second experimental group had 15 samples with a 1% concentration gradient of proteoglycan (range, 10–24%), with a higher water content compared with the first group. The third experimental group contained 20 samples with a concentration gradient of 1% proteoglycan (range, 10–29%), with 75% water content. All of the *in vitro* models were scanned using a 3.0T GE MR. To analyze the correlation between the proteoglycan content of the *in vitro* model and the T1ρ value, we investigated the feasibility and stability of modeling.

Results: There was no correlation between the *in vitro* model proteoglycan concentration and T1ρ value in the first experimental group; however, there was a significant negative correlation between the proteoglycan concentration and T1ρ value in the second experimental group ($Y=-3.02X+131.8$, $R^2=0.852$, $P<0.05$). In the third experimental group, the proteoglycan concentration was significantly positively correlated with T1ρ value ($Y=3.05X+11.99$, $R^2=0.834$, $P<0.05$). The comparison of the T1ρ values in the third experimental group before and 3 months after yielded an intraclass correlation coefficient value of 0.980, indicating that the biochemical components in the third experimental group were still stable after 3 months of storage. The slope of the regression equation between the Pfirrmann grading and T1ρ value in the third experimental group was not statistically different from the volunteer group ($F=0.54$, $P=0.814$), suggesting that the lumbar disc nucleus pulposus tissue of *in vitro* model samples fitted well with the volunteer group.

Conclusions: In this experiment, we successfully constructed an *in vitro* model of nucleus pulposus tissue proteoglycan that can be used for the quantitative evaluation of the MR-T1ρ imaging.

Keywords: Lumbar disc degeneration; nucleus pulposus tissue; proteoglycan; *in vitro* model; magnetic resonance T1rho

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Introduction

Low back pain is common, and in severe cases, can affects people's quality of life and ability to work (1). Worsening lumbar disc degeneration can cause lumbar disc herniation. Therefore, it important to detect and treat degeneration of the lumbar intervertebral disc as early as possible in the clinical setting to delay or even reverse the degeneration of the intervertebral disc and prevent further deterioration.

The accurate diagnosis of early degeneration of the lumbar intervertebral disc has important clinical value and significance. Previous studies have confirmed that the early degeneration of lumbar intervertebral disc is first reflected in the content change of proteoglycans in the nucleus pulposus tissue (2); however, the non-invasive imaging methods currently used cannot quantitatively detect proteoglycans. Due to the tissue structure of the lumbar intervertebral disc, the early diagnosis of degeneration cannot be confirmed by needle biopsy. Magnetic resonance imaging (MRI) is one of the most commonly used imaging techniques for diagnosing soft tissue diseases. Many scholars have reported that magnetic resonance T1rho (MR-T1ρ) imaging technology can quantify the changes of proteoglycan content in the nucleus pulposus tissue of early lumbar disc degeneration (3,4), and that it is more sensitive than conventional magnetic resonance T2 weighted image (MR-T2WI) imaging and T2-mapping imaging. In addition, some scholars have found that when studying degeneration of the lumbar intervertebral disc in humans and cadavers, MR-T1ρ imaging is more sensitive than MR-T2WI imaging in the early degeneration of the lumbar intervertebral disc (5). The proteoglycan concentration in the lumbar intervertebral disc nucleus pulposus tissue has been analyzed in many studies, which have found that the proteoglycan concentration has a good and positive correlation with the T1ρ relaxation time value (6-8).

However, when reviewing studies using MR-T1ρ imaging to quantitatively assess early lumbar disc degeneration, we found that in the lumbar disc nucleus pulposus tissue with the same Pfirrmann degeneration grade, significant differences in the MR-T1ρ relaxation time value have been reported (9). When we studied the correlation between the proteoglycan content in the nucleus pulposus tissue of the natural degeneration and the MR-T1ρ value of the rhesus monkeys in the early stage, we found significant differences in the MR-T1ρ value when the same lumbar disc nucleus pulposus tissue was detected on different magnetic resonance equipment. Therefore, if the MR-T1ρ value measured by the magnetic resonance equipment is directly used to quantitatively evaluate the proteoglycan content of the human lumbar intervertebral disc nucleus pulposus, the MR-T1ρ value data obtained by different examination equipment of intervertebral discs with similar degrees of degeneration will also have differences, which cannot meet the requirements of a clinically accurate diagnosis of early degeneration of lumbar intervertebral disc. Therefore, when using MR-T1ρ imaging technology to quantitatively evaluate the degeneration of the lumbar intervertebral disc, an *in vitro* calibration model is required on clinical application to solve the detection error of the magnetic resonance T1rho (MR-T1ρ) sequence on different machines. In the present study, we constructed a series of *in vitro* models of nucleus pulposus tissue with different proteoglycan concentrations to measure the MR-T1ρ relaxation time of the *in vitro* models. We determined if there were any similar characteristics between the *in vitro* nucleus pulposus tissue models and the human degenerated lumbar disc nucleus pulposus tissue, providing theoretical data for the establishment of a standardized mathematical model of degenerated lumbar intervertebral disc nucleus pulposus tissue and MR-T1ρ relaxation time value in the future. We present the following article in accordance with the MDAR reporting checklist (available at https://dx.doi.org/10.21037/atm-21-4297).

Methods

Proteoglycan concentration in the in vitro model of nucleus pulposus tissue

The 75% proportion was selected as the water content of the *in vitro* model, which was 2.25 g. The concentration of proteoglycan and the content of type II collagen fibers were set. Three groups were involved in the present study. In the first group, the water content and type II collagen fibers (West Asia Reagent Company, Chengdu, China) were set to 2.25 and 0.3 g, respectively. The concentration range of proteoglycan (West Asia Reagent Company) was set to 5–35%, with a concentration gradient of 5%. The 2 control groups (water with proteoglycan and water with type II collagen) and all of the *in vitro* models in this group were not sterilized after construction. To ensure uniformity in the *in vitro* model, the water content in the second group of the *in vitro* models was increased from 2.25 to 3 g, the content of type II collagen fiber remained 0.3 g, and the proteoglycan concentration range was set to 10–24%,

with a concentration gradient of 1%. After the model was constructed, it needed to be sterilized by ultraviolet light and sealed and stored at a low temperature (10–15 ℃). The water content and type II collagen fibers in the third group of *in vitro* models was the same as that in the first group; the proteoglycan concentration range was set to 10–29%, with a concentration gradient of 1%. After the *in vitro* model in the third experimental group was constructed, it also needed to be sterilized by ultraviolet light and sealed and stored at a low temperature (10–15 ℃).

Methods of constructing an in vitro model of nucleus pulposus tissue

First, the reagent bottle was rinsed with deionized water and then sterilized and dried. The required proteoglycan and type II collagen were weighed in a sterile laboratory, and the materials were put into the reagent bottle. The required amount of water was added using a micropipette. The materials were stirred with a magnetic stirrer (Dalong Xingchuang, Beijing, China), by ultrasonic mixing, heating and shaking to mix the molding material into a gel-like material, and finally sterilized by ultraviolet light and stored at a low temperature. The model samples in the first group were modeled with a magnetic stirrer for 30 min, and the samples were not sterilized by ultraviolet rays or stored at low temperatures after modeling. The model samples in the second group were modeled by ultrasonic stirring for 30 min. After modeling, the samples were sterilized by ultraviolet light and stored at a low temperature (10–15 ℃). The model samples in the third group were heated and shaken for 24 h (temperature: 60 ℃; the temperature was automatically increased from room temperature to 60 ℃ within 24 h), and then ultrasonically stirred for 30 min for modeling. After modeling, the samples were sterilized by ultraviolet light and stored at a low temperature (10–15 ℃). When the *in vitro* model failed to form a gel-like substance or there was obvious stratification, the *in vitro* model construction was considered to have failed.

Acquisition of magnetic resonance image

The clinical 3.0T GE magnetic resonance scanner (Discovery MR 750w 3.0T; GE Medical System, Boston, Massachusetts, USA) was used to scan the T1ρ sequence of the *in vitro* model. Two researchers who did not participate in the modeling selected the region of interest (ROI). Finally, the post-processing software provided by GE was employed to calculate the T1ρ relaxation time value. The human lumbar intervertebral disc was also scanned by T1ρ sequence with the same parameter, and its T1ρ relaxation time value was measured. The human lumbar intervertebral disc was scanned using the conventional T2WI sequence and then graded by a professional according to the Pfirrmann classification.

The main parameters of the MR-T1ρ imaging technology included pulse sequence, fast spin echo (10, 30, and 60 ms), repetition time: 80 ms, echo time: 8 ms, field of view: 160 mm × 100 mm, frequency (pixel bandwidth): 500 Hz, voxel size: 0.59 mm × 1.17 mm, Slice thickness: 3 mm, interslice gap: 0 mm, number of slices: 5–7, and acquisition time (min): 4 min. According to the volume of the *in vitro* model samples, the MR-T1ρ sequence scan in the first group was scanned by small size flexible-joint phased array coils with 8-channel. The second and third groups were scanned with small size flexible-joint phased array coils with 16-channel.

Image post-processing

A double-blind experimental method was adopted to obtain the ROI. Drawing of the ROI area was done by 2researchers. The basic conditions of selecting the ROI area were as follows (*Figure 1A*,*1B*): (I) the participator should choose at least 2 levels closing to the center. Edge area and defect part should be avoided to make the value of each region have a uniform representation; (II) the shape of the ROI was generally elliptical, and each ROI drawn was similar in size, and (III) the T1ρ relaxation time value of the ROI was measured and recorded 3 times for each sample in the *in vitro* model, and the average value was taken as the final statistical analysis data. The T1ρ value was calculated using a previously reported method (10). After being stored at a low temperature of 10–15 ℃ for 3 months, the third group of *in vitro* model samples was rescanned by MR-T1ρ with the same scanning parameters.

Statistical analysis

All statistical data were analyzed using SPSS version 25.0 (SPSS Inc. Chicago, IL, USA). Measurement data were expressed as mean ± standard deviation. To test whether the T1ρ relaxation time value of the *in vitro* model in line with normal distribution, the Pearson correlation coefficient was used to analyze the correlation between the *in vitro* model proteoglycan concentration and the T1ρ value. The

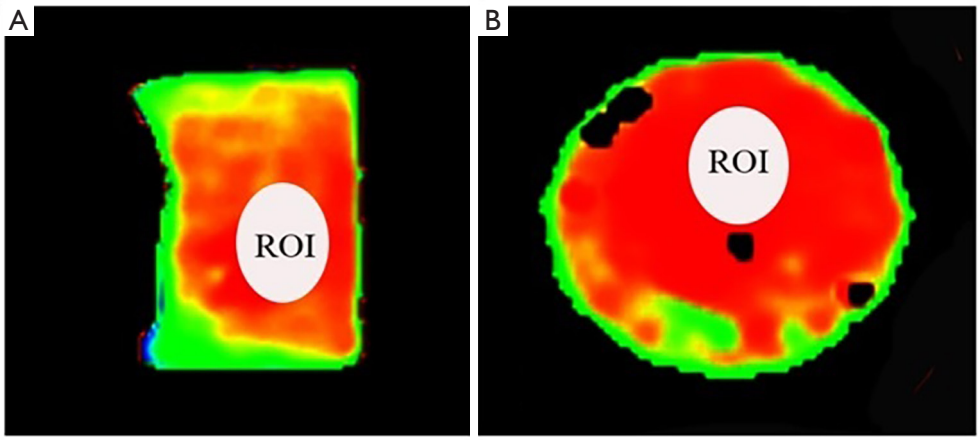

Figure 1 Schematic diagram of measurement of the *in vitro* model magnetic resonance T1rho (MR-T1ρ) relaxation time value. (A) Schematic diagram of the region of interest (ROI) of the coronal position of the *in vitro* model. (B) Schematic diagram of the horizontal position of the ROI of the *in vitro* model.

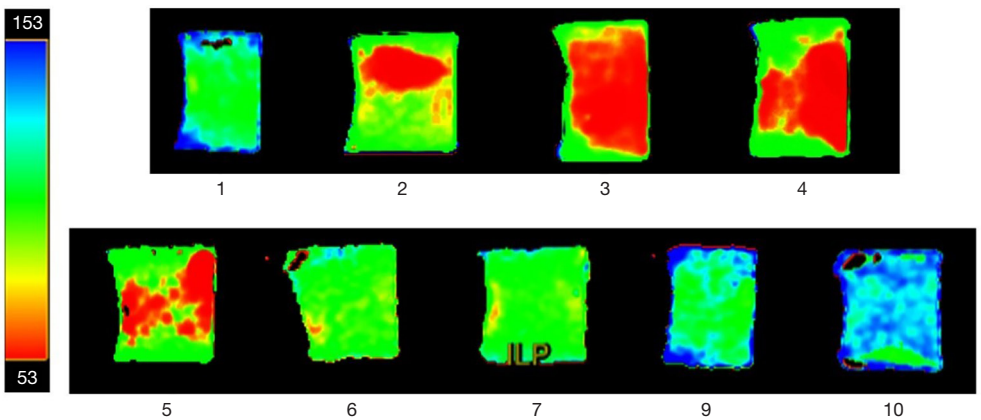

Figure 2 Magnetic resonance T1rho (MR-T1C) pseudo-color image of *in vitro* model samples in the first experimental group in the coronal position. There were defective areas in small amount of the samples, as indicated by the uneven color distribution.

slope of the regression equation between the T1ρ value and Pfirrmann grading of the *in vitro* model and volunteer lumbar disc nucleus pulposus tissue was analyzed by covariance analysis. The consistency test and evaluation of the T1ρ value of the same batch of *in vitro* model samples at different detection times were analyzed by intraclass correlation coefficient (ICC). $P<0.05$ indicated statistical difference.

Results

MR-T1ρ imaging of the in vitro model and its T1ρ relaxation time value

Of the 10 *in vitro* model samples in the first experimental group, sample 8 failed to form, so the success rate of the model making was 90%. The MR-T1ρ images of the *in vitro* model are shown in *Figure 2*. The biochemical compositions of each sample successfully modeled and their corresponding T1ρ values are shown in *Table 1*. Of these, *in vitro* model group 1 was used as the control group. Type II collagen was not added to this group, and its T1ρ value was 200 ms. Proteoglycan was not added to control group 10, with its T1ρ value was 306.1 ms. T1ρ values of *in vitro* models 2–9 in the experimental group deviated significantly from the control group.

Of the 15 *in vitro* model samples in the second experimental group, the success rate of model making was

Table 1 Biochemical components of the 9 model samples in the first experimental group and their T1rho (T1ρ) relaxation time values

Serial number	Proteoglycan (g)	Type II collagen (g)	Water (g)	Proteoglycan concentration (%)	T1ρ value (ms)
1	0.25	0	2.25	10	200.0±4.3
2	0.13	0.3	2.25	5	34.3±2.0
3	0.28	0.3	2.25	10	75.6±4.6
4	0.45	0.3	2.25	15	45.0±2.2
5	0.63	0.3	2.25	20	54.3±2.2
6	0.85	0.3	2.25	25	73.4±4.0
7	1.10	0.3	2.25	30	63.9±4.1
9	1.37	0.3	2.25	35	102.2±2.1
10	0	0.3	2.25	0	306.1±5.0

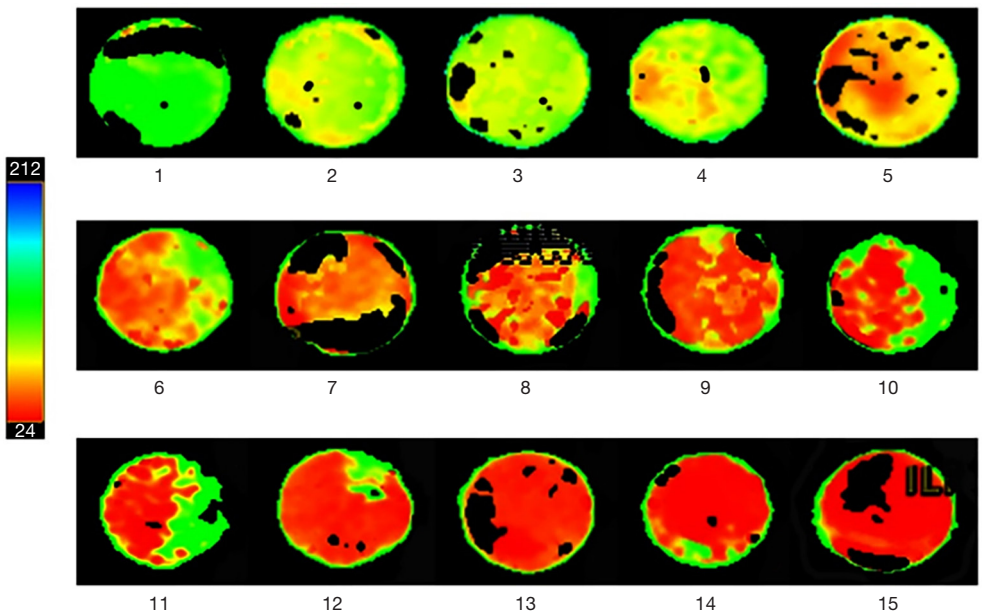

Figure 3 Magnetic resonance T1rho (MR-T1ρ) sequence pseudo-color image of *in vitro* model samples in the second experimental group in the horizontal position.

100%. The MR-T1ρ images of the *in vitro* model sample are shown in *Figure 3*, and small, spot-like or patchy defects could be seen in the model sample. The biochemical compositions of each sample and their corresponding T1ρ values are shown in *Table 2*.

Of the 20 *in vitro* model samples in the third experimental group, 5 samples were not modeled, so the success rate of the model-making process was 75%. The samples modeled successfully were tested twice. The MR-T1ρ images of the model samples tested for the first time are shown in *Figure 4*. No defect area was seen inside the model. The model samples tested for the second time were stored at 10–15 ℃ for 3 months, and then MR-T1ρ imaging was re-performed, with MR-T1ρ images shown in *Figure 5*. Of these, 4 model samples had small patchy defects inside. The biochemical components and their T1ρ values of 15 successful *in vitro* model samples tested twice are shown in *Table 3*.

The ICC was further used to test the consistency of the 2 T1ρ values before and after the cold storing of each sample.

Table 2 Biochemical components of the model samples in the second experimental group and their T1rho (T1ρ) relaxation time values

Serial number	Proteoglycan (g)	Type II collagen (g)	Water (g)	Proteoglycan concentration (%)	T1ρ value (ms)
1	0.367	0.3	3	10	98.85±2.6
2	0.403	0.3	3	11	97.81±2.6
3	0.440	0.3	3	12	91.61±1.1
4	0.477	0.3	3	13	84.10±1.8
5	0.513	0.3	3	14	101.91±3.4
6	0.550	0.3	3	15	91.74±1.6
7	0.587	0.3	3	16	76.85±0.5
8	0.623	0.3	3	17	79.09±1.4
9	0.660	0.3	3	18	75.22±3.8
10	0.697	0.3	3	19	82.16±1.6
11	0.733	0.3	3	20	73.09±0.4
12	0.770	0.3	3	21	71.73±1.9
13	0.807	0.3	3	22	63.44±1.2
14	0.843	0.3	3	23	58.64±3.0
15	0.880	0.3	3	24	54.09±5.5

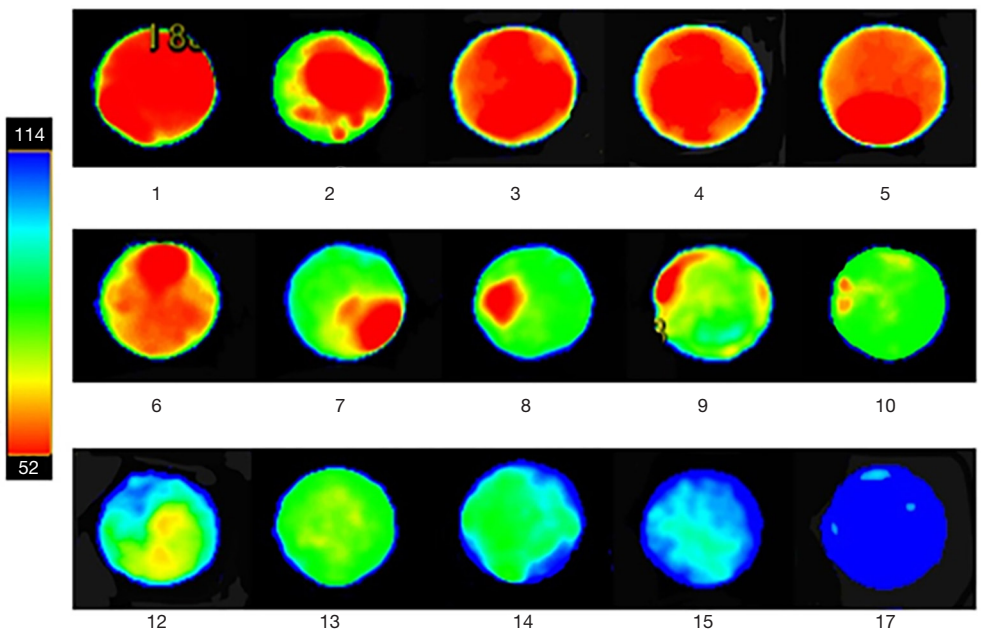

Figure 4 Magnetic resonance T1rho (MR-T1ρ) sequence pseudo-color image of *in vitro* model samples in the third experimental group in the horizontal position.

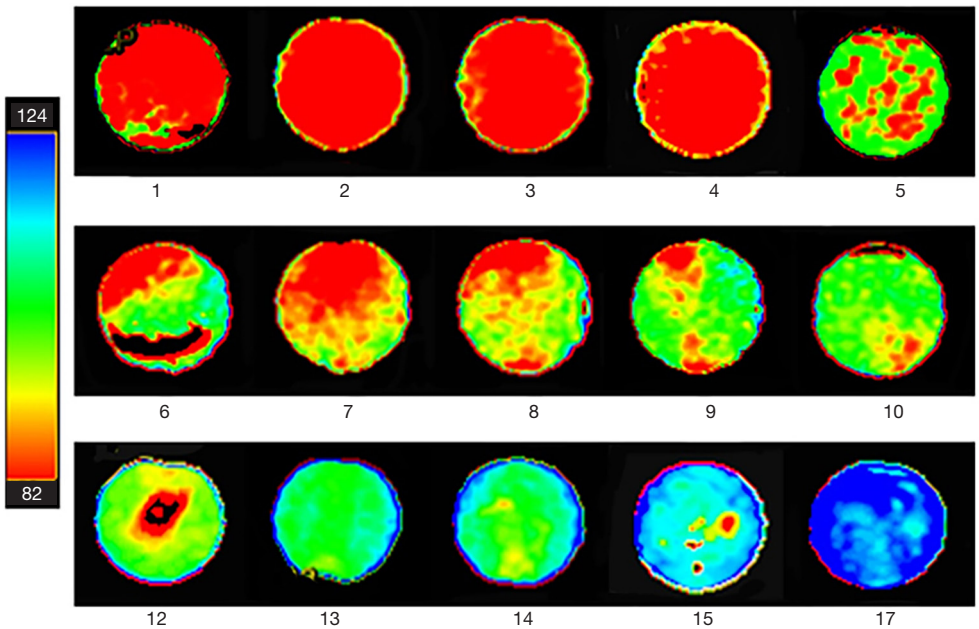

Figure 5 Magnetic resonance T1rho (MR-T1ρ) sequence pseudo-color image after 3 months modeling of the *in vitro* model samples in the third experimental group in the horizontal position.

Table 3 Biochemical components of the 15 sample models in the third experimental group and their 2 T1rho (T1ρ) relaxation time values

Serial number	Proteoglycan (g)	Type II collagen (g)	Water (g)	Proteoglycan concentration (%)	T1ρ values of the first test (ms)	T1ρ values of the second test (ms)
1	0.283	0.3	2.25	10	44.81±2.6	51.90±3.2
2	0.315	0.3	2.25	11	48.97±3.4	48.51±1.6
3	0.348	0.3	2.25	12	51.07±1.5	51.70±2.2
4	0.380	0.3	2.25	13	51.63±3.4	52.65±2.7
5	0.415	0.3	2.25	14	53.16±2.4	54.30±2.0
6	0.449	0.3	2.25	15	58.71±1.9	61.23±1.3
7	0.486	0.3	2.25	16	63.90±1.3	69.95±2.8
8	0.523	0.3	2.25	17	61.65±1.6	63.33±2.3
9	0.560	0.3	2.25	18	62.66±4.3	68.36±3.9
10	0.598	0.3	2.25	19	71.70±2.9	73.70±1.2
12	0.638	0.3	2.25	21	67.24±3.2	69.36±3.9
13	0.678	0.3	2.25	22	61.46±2.3	73.69±2.1
14	0.719	0.3	2.25	23	83.60±3.2	84.36±3.3
16	0.761	0.3	2.25	25	92.83±0.3	93.37±0.8
17	0.805	0.3	2.25	26	105.5±6.3	109.60±7.9

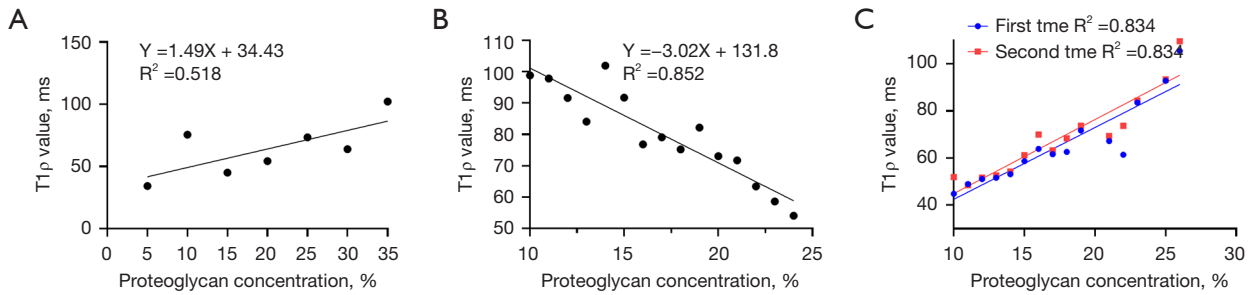

Figure 6 Correlation analysis between the proteoglycan concentration and T1rho (T1ρ) values of the model samples in the experimental groups. (A) Scatterplot and regression line of the proteoglycan concentration of the *in vitro* model samples in the first experimental group and their T1ρ values; (B) scatterplot and regression line of the proteoglycan concentration of the model samples in the second experimental group and their T1ρ values; (C) scatterplot and the regression line of the proteoglycan concentration and T1ρ values for the first and second measurements of the model samples in the third experimental group.

The results showed that the ICC of the T1ρ values of the 2 scans was 0.980, which was greater than 0.75, suggesting that the biochemical components of the *in vitro* model samples in the third experimental group were still relatively stable after being stored for 3 months.

Correlation between in vitro model proteoglycan concentration and T1ρ value of the 3 groups

Plotting was done by Pearson linear regression analysis, and the T1ρ relaxation time values of samples 2–9 in the first group were found to be normally distributed. The results showed that there was no correlation between the *in vitro* model proteoglycan concentration and T1ρ values (Y=1.49X+34.43, R^2=0.518, P>0.05) (*Figure 6A*). The T1ρ values of the 15 samples in the second group were normally distributed, as plotted by Pearson linear regression analysis. The results showed that there was a distinctly negative correlation between the proteoglycan concentration of the *in vitro* model samples and their T1ρ values (Y=–3.02X+131.8, R^2=0.852, P<0.05) (*Figure 6B*).

The T1ρ values of the 15 *in vitro* model samples in the third group were normally distributed, as plotted by Pearson linear regression analysis. The results showed that there was a distinctly positive correlation between the proteoglycan concentration in the first *in vitro* model samples and the T1ρ values (Y=3.05X+11.99, R^2=0.834, P<0.05) (*Figure 6C*). The comparison of the slope of the regression equation between the proteoglycan concentration in the third experimental group in the *in vitro* model sample and the first measurement, as well as the second measurement, showed that there was no significant difference between them (F=1.27, P=0.270), with a good degree of fit. This finding indicated that the biochemical components of the model samples were still relatively stable after being sealed and cryopreserved.

MR-T1ρ correlation analysis of the third experimental group in the in vitro model and lumbar intervertebral disc of the volunteers

The MR-T1ρ imaging of the *in vitro* model samples were compared with the human lumbar intervertebral discs of volunteers. In this experiment, the MR-T1ρ sequence with the same parameters was used to scan the lumbar intervertebral discs of 4 adult volunteers with no low back pain, and the T1ρ values of the nucleus pulposus tissue of their intervertebral discs were measured (*Table 4*). T2WI sequence scanning was applied to obtain T2WI images and Pfirrmann grading evaluation to establish the regression equation between the Pfirrmann grading of the lumbar disc nucleus pulposus tissues of volunteers and their T1ρ values (Y=–10.43X+75.60, R^2=0.731) (*Table 5*, *Figures 7,8*).

To evaluate the fit degree of the T1ρ values between the third experimental group model samples and the lumbar intervertebral disc nucleus pulposus tissues of volunteers, the *in vitro* model sample proteoglycan concentration of 8–10% was set as Pfirrmann III, 11–16% as Pfirrmann II, and 17–20% as Pfirrmann I. The regression equation between the Pfirrmann grading and T1ρ values of the *in vitro* model samples in the third experimental group was obtained (Y=–8.69X+133.6, R^2=0.765) (*Figure 8*). The covariance comparison of the slope of the regression

Table 4 T1rho (T1ρ) values of lumbar intervertebral disc nucleus pulposus in 4 volunteers with no low back pain (ms)

Lumbar disc	No. 1 T1ρ value (ms)	No. 2 T1ρ value (ms)	No. 3 T1ρ value (ms)	No. 4 T1ρ value (ms)
Lumbar 1/2	123.8±0.5	118.8±0.6	124.7±1.0	124.3±0.7
Lumbar 2/3	125.6±0.4	110.3±0.6	116.9±0.4	117.8±0.5
Lumbar 3/4	119.9±0.5	112.6±0.7	112.3±0.5	119.3±0.2
Lumbar 4/5	77.6±0.7	82.7±0.2	101.3±0.7	86.3±0.6
Lumbar 5/sacral 1	85.8±0.7	69.8±0.5	97.6±0.5	73.7±0.4

Table 5 Pfirrmann grading of lumbar intervertebral discs of 4 volunteers with no low back pain

Lumbar disc	No. 1	No. 2	No. 3	No. 4
Lumbar 1/2	I	II	I	I
Lumbar 2/3	I	II	II	II
Lumbar 3/4	II	III	II	II
Lumbar 4/5	IV	IV	III	IV
Lumbar 5/sacral 1	V	V	IV	V

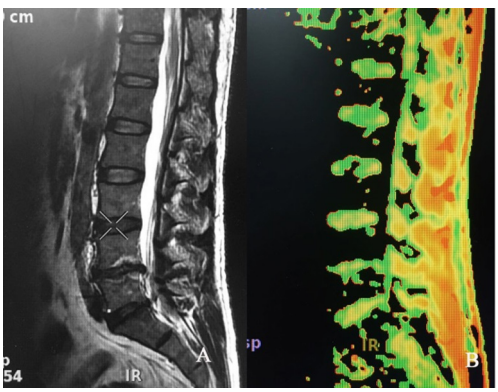

Figure 7 Sequence images. (A) Magnetic resonance T2 weighted image (MR-T2WI) sequence images of volunteers' lumbar intervertebral discs; (B) Magnetic resonance T1rho (MR-T1ρ) sequence pseudo-color images.

equation between the Pfirrmann grading and T1ρ values between the *in vitro* model samples in the third experimental group and the lumbar intervertebral disc nucleus pulposus tissues of volunteers indicated no significant differences between them (F=0.54, P=0.814), suggesting that the slope of regression equation between the Pfirrmann grading and T1ρ value in the *in vitro* model samples and lumbar disc nucleus tissues of volunteers was a good fit.

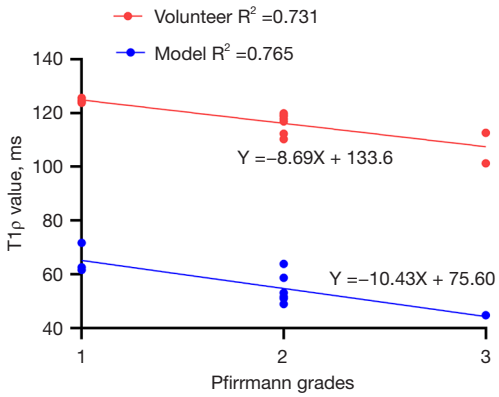

Figure 8 Scatterplot and regression line of the regression equation between the Pfirrmann grading and T1rho (T1ρ) value of the model samples in the third experimental group and volunteers.

Discussion

Approximately 80% of people worldwide will experience low back pain during their lifetime, and 5% will develop chronic low back pain, which affects quality of life and ability to work (11). Degeneration of the lumbar intervertebral disc is the main cause of low back pain. China has a large, aging population, so early diagnosis and the prevention and treatment of this disease it is of great economic.

The most accurate diagnostic method for early degeneration of lumbar intervertebral disc is to directly obtain the nucleus pulposus tissue to quantitatively measure the biochemical components, but this method is associated with invasive injury to the intervertebral disc, which will aggravate the degeneration of the intervertebral disc, rendering it unsuitable for clinical application (12). Studies have shown that the main biochemical change in the early degeneration of the intervertebral disc is the reduction of proteoglycan in the nucleus pulposus tissue (13). Therefore, there is a clinical need for a non-invasive, quantitative

method to detect the proteoglycan content in the nucleus pulposus tissue of the intervertebral disc.

Lumbar X-rays and computed tomography scans cannot diagnose early disc degeneration, and conventional magnetic resonance T2WI sequencing cannot quantitatively evaluate the degree of lumbar disc degeneration. At present, only MR-T1ρ and T2-mapping imaging technology can be used to quantitatively measure early lumbar disc degeneration without invasive injury (12). Studies have shown that using MR-T1ρ sequence to quantitatively evaluate nucleus pulposus tissue proteoglycan is superior to other magnetic resonance sequences (14). Because MR-T1ρ imaging technology is sensitive to the low-frequency movement of macromolecular substances, and the proteoglycan molecules in the nucleus pulposus tissue of the intervertebral disc are macromolecular substances, MR-T1ρ technology can be used to quantitatively evaluate the proteoglycan content in the nucleus pulposus tissue of the intervertebral disc (15,16). Previous studies have shown that the T1ρ relaxation time is significantly and positively correlated with the proteoglycan content of human or animal lumbar intervertebral discs (17,18). Our research group has researched the natural degeneration of lumbar intervertebral discs in rhesus monkeys, and confirmed that the proteoglycan content of the lumbar disc nucleus pulposus of rhesus monkeys is positively correlated with the T1ρ value. However, we also found that the T1ρ value of the lumbar intervertebral disc from the same rhesus monkey was significantly different when scanned by different MRI equipment. Therefore, the T1ρ value cannot be directly used to accurately diagnose the early degeneration of the human lumbar intervertebral disc without calibration.

The quantification and calibration of T1ρ values are technical difficulties in the clinical setting (19), and some methods have been reported to calibrate T1ρ values to increase the detection rate of diseases (20-22). In the present study, we set up *in vitro* model samples with different proteoglycan content or water content to simulate different degenerated nucleus pulposus tissues. In the *in vitro* model in the first experimental group, the results showed that more defective areas were seen in the MR-T1ρ image of the modeled samples. After changing the experimental conditions, some spots or flaky defect areas still could still be seen from the MR-T1ρ image of the *in vitro* model in the second experimental group. After another conditional improvement, the MR-T1ρ image showed no obvious defect area in the modeled samples in the third experimental group, and the proteoglycan concentration was positively correlated with the T1ρ value. The *in vitro* model samples in the third group underwent MR-T1ρ imaging re-scanning after being sterilized by ultraviolet rays and sealed at a low temperature (10–15 ℃) for 3 months. According to the ICC value, the biochemical components of the model samples were still stable after a long period of storage. The results of these 3 groups confirmed that the ideal *in vitro* model with stable biochemical components needs a higher water content and the cryopreserved samples should be aseptically seal.

We selected 4 adult volunteers with no low back pain to perform the T1ρ sequence scan and T2WI sequence scan with the same parameters, and evaluated the Pfirrmann grading of their lumbar intervertebral discs. The results showed that there were no obvious differences between the slope of the regression equation between the Pfirrmann grading and T1ρ value of the *in vitro* model samples in the third experimental group and the lumbar intervertebral disc tissue of volunteers, suggesting that this model has a good degree of fit with the degeneration of the nucleus pulposus tissue of human lumbar intervertebral disc.

Conclusions

In this experiment, we successfully constructed an *in vitro* model of MR-T1ρ to quantitatively evaluate the proteoglycans of nucleus pulposus tissue. However, to accurately quantify the proteoglycan content of human lumbar intervertebral disc nucleus pulposus by clinical MR-T1ρ imaging technology, large samples are required to establish an *in vitro* model database and post-processing platform to accurately detect theMR-T1ρ value of human lumbar intervertebral disc nucleus pulposus tissue, and to provide a scientific method for the accurate diagnosis of early degeneration of the lumbar intervertebral disc.

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Footnote

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