Magnetic resonance imaging of the zone of calcified cartilage in the knee joint using 3-dimensional ultrashort echo time cones sequences

Jin Liu1, Yang Wei2, Ya-Jun Ma3, Yan-Chun Zhu4, Quan Zhou1, Ying-Hua Zhao1,3

1Department of Radiology, Third Affiliated Hospital of Southern Medical University (Academy of Orthopedics, Guangdong Province), Guangzhou, Guangdong 510630, China; 2Guangdong Provincial Key Laboratory of Medical Image Processing, School of Biomedical Engineering, Southern Medical University, Guangzhou, Guangdong 510515, China; 3Department of Radiology, University of California, San Diego, San Diego, CA, USA.

Abstract

Background: The zone of calcified cartilage (ZCC) plays an important role in the pathogenesis of osteoarthritis (OA) but has never been imaged in vivo with magnetic resonance (MR) imaging techniques. We investigated the feasibility of direct imaging of the ZCC in both cadaveric whole knee specimens and in vivo healthy knees using a 3-dimensional ultrashort echo time cones (3D UTE-Cones) sequence on a clinical 3T scanner.

Methods: In all, 12 cadaveric knee joints and 10 in vivo healthy were collected. At a 3T MR scanner with an 8-channel knee coil, a fat-saturated 3D dual-echo UTE-Cones sequence was used to image the ZCC, following with a short rectangular pulse excitation and 3D spiral sampling with conical view ordering. The regions of interests (ROIs) were delineated by a blinded observer. Single-component T2* and T2 values were calculated from fat-saturated 3D dual-echo UTE-Cones and a Carr-Purcell-Meiboom-Gill (T2 CPMG) data using a semi-automated MATLAB code.

Results: The single-exponential fitting curve of ZCC was accurately obtained with R2 of 0.989. For keen joint samples, the ZCC has a short T2* ranging from 0.62 to 2.53 ms, with the mean ± standard deviation (SD) of 1.49 ± 0.66 ms, and with 95% confidence intervals (CI) of 1.20–1.78 ms. For volunteers, the short T2* ranges from 0.93 to 3.52 ms, with the mean ± SD of 2.09 ± 0.56 ms, and the 95% CI is 1.43 to 2.74 ms in ZCC.

Conclusions: The high-resolution 3D UTE-Cones sequence might be used to directly image ZCC in the human knee joint on a clinical 3T scanner with a scan time of more than 10 min. Using this non-invasive technique, the T2* relaxation time of the ZCC can be further detected.

Keywords: Ultrashort echo time; T2* relaxation time; Zone of calcified cartilage; Articular cartilage

Introduction

In all over the world, there are approximately 360 million people affected by osteoarthritis (OA), which occurs in 50% elder people aged 60 years.[1] Early-stage of OA is clinically silent prior to structural changes. Articular cartilage has a layered structure which can be divided into four zones: the superficial zone, the transitional zone, the radial zone and the zone of calcified cartilage (ZCC).[2] The ZCC forms an important interface between articular cartilage and bone for both solute transportation and force transmission.[3] It has been proved that age-related factors of ZCC could contribute the degeneration adjacent noncalcified cartilage.[4] At these earliest stages, ZCC was active in OA, accompanying with changes in the mineral content and thickness of ZCC.[5] Micro-cracking and vascular invasion of the ZCC have been implicated in the pathogenesis of OA.[6] The articular surface and matrix remain intact, and pathological changes have the highest likelihood of reversal in the early stages of OA.[7] Hence, the direct imaging and quantification of the ZCC in the whole knee may be useful for early diagnosis and monitoring treatment of OA.

It was well known that using magnetic resonance imaging (MRI), knee articular cartilage could be effectively evaluated in morphology, particularly in the surface irregularity and/or loss of cartilage thickness, with sensitivities of 93% to 94%.[8] Unfortunately, due to the high mineral content, the ZCC has intrinsically short T2* relaxation time, which makes it invisible in MRI. More than 90% OA patients are treated with invasive procedures, such as joint replacement or debridement, and most of them have OA of the knee. Hence, it is important and urgent to develop non-invasive and less invasive diagnostic tools as well as treatment options for OA.
characteristics with estimated T2* values of 1.0 to 3.3 ms. So conventional clinical MRI sequences have no or low signals for ZCC imaging. With the advent of ultrashort echo time (UTE) MR imaging, using a half-slice selection and variable-rate selective excitation (VERSE), and rapid transmit/receive (T/R) switching to get a nominal TE as short as 8 ms, which is 100 to 1000 times shorter than those routinely available on clinical MR systems, UTE imaging minimizes short-T2 signal decay during signal acquisition, which is able to show the short-T2 contrast in tissues including cortical bone, menisci, ligaments, tendons, and calcification. Recently, UTE imaging has been proven to describe ZCC lesions better than traditional MR sequences. Based on a multi-echo interleaved variable TE UTE acquisition, a spectroscopic UTE imaging (UTESI) estimated T2* values in ZCC to be between 1 and 2 ms in volunteer and cadaveric whole knee joints. A 2-dimensional (2D) dual inversion recovery ultrashort echo time (DIR-UTE) MR techniques have also demonstrated the ZCC ex vivo. Moreover, correlations between quantitative MR parameters and biochemical, and microstructural changes in ZCC have also been investigated, and they found that T2* values could be used to monitor content and microstructural changes in collagen matrix. However, it is still challenging to image and quantify the ZCC in the whole knee with UTE MR imaging techniques because it is quite time-consuming for high spatial resolution imaging which is needed for imaging ZCC with a very thin layer (on the order of 0.1 mm). Preview studies have demonstrated that 3-dimensional (3D) UTE Cones multi-echo sequence is a quite advanced UTE MR imaging sequence for quantitative.

T2* assessment of short T2 tissues, which is fast and has a high signal-to-noise ratio (SNR) efficiency because of the special 3D anisotropic Cones k-space trajectory. In this study, we aimed to develop a 3D UTE Cones multi-echo imaging protocol for whole knee ZCC imaging on a clinical 3T scanner. T2* values were also used to quantify the ZCC in both human cadaveric knee joint specimens and in vivo healthy knees.

Methods

Ethical approval

The volunteer was informed with his written consent, and this study was approved by the Ethics Committee in the Third Affiliated Hospital of Southern Medical University and University of California, San Diego.

Study design and participants

In total, 12 cadaveric knee joints [5 males, 7 females; mean age of 47.85 ± 22.21 years and age range of (28, 81) years] and 10 in vivo healthy knee from 10 volunteers [6 males, 4 females; mean age of 32.90 ± 8.39 years and age range of (26, 49) years] were imaged in this study. Knee samples were obtained in accordance with protocols approved by the Committee for the Oversight of Research for the Dead (CORID) and the Institutional Review Board (IRB). All the samples in this study only underwent one single free-thaw cycle with a frozen degree of -80°C. A fat saturated dual-echo 3D UTE Cones sequence was used to image the ZCC on a 3T whole-body GE scanner (GE Healthcare Technologies, Milwaukee, WI, USA) with a maximum gradient performance of 50 and 200 mT/m. An 8-channel transmit-receive knee coil was used for both RF transmission and signal acquisition. The fat-saturated 3D UTE Cones sequence employed a short rectangular pulse excitation (hard pulse) followed by 3D spiral trajectories with conical view ordering to provide anisotropic imaging (high in-plane resolution and thicker slices) [Figure 1]. This technique allows for fast volumetric imaging of the knee joint.

The imaging parameters included: FOV = 8 cm × 8 cm × 3 cm, matrix = 256 × 256 × 30, flip angle = 10°, bandwidth = 125 kHz, TR = 90 ms; for the cadaveric knee joint specimens, six groups of dual-echo UTE-Cones sequences with different TEs (0.1/6.6, 0.2/6.6, 0.4/6.6, 0.6/6.6 and 2.2/6.6 ms) were used for T2* measurement of ZCC and scan time was 20 min; for the volunteer knee joint, the scan time of the dual-echo scan with 3 different echo time groups (i.e., 0.032/4.3, 0.3/6.6, and 0.8/8.8 ms) was 10.6 min. Echo subtraction image (i.e., the first echo image substrating the second echo image) was used to generate high short T2
contrast to find the region of interest of ZCC and other soft tissue calcification for $T_2^*$ measurement. For comparison, conventional MR sequences were also performed, including a proton-density weighted fast spin echo (PD-FSE) sequence, a T1-weighted fast spin echo (T1-FSE), a fat saturation T2-weighted fast spin echo (T2-FS-FSE), and a Carr-Purcell-Meiboom-Gill (T2 CPMG) sequence for T2 measurement.

**Image analysis**

Single-component $T_2^*$ and T2 values analysis were performed using a semi-automated MATLAB (The Mathworks Inc., Natick, MA, USA) code developed in-house as previously described.[20] The regions of interests (ROIs) were drawn on ZCC in volunteer knee joints and samples, respectively. To minimize partial volume effects, the ROIs were placed within the inner edge of ZCC. As shown in Figure 2C, from which the average signal was used for fitting. Mean UTE-$T_2^*$ values for ROIs were recorded for analysis, and evaluated by a musculoskeletal radiologist of 20-year experiences.

$T_2^*$ values were described as the mean, standard deviation (SD), 95% confidence intervals (CI) range and standard error of the mean (SEM) for the normal ZCC.

$T_2^*$ or T2 was fitted using a mono-exponential model as shown in the Eq. (1).

$$ s = ae^{-TE/T_2} $$  \hspace{1cm} (1)

![Figure 2](image_url) A 63-year-old female cadaveric knee joint specimen. MR imaging of articular cartilages using CPMG-T2 (A) and 3D fat saturated UTE Cones sequences (C). The clinical FSE and CPMG sequences show a signal void for the ZCC. A single-component exponential fitting curves of T2 values show 35.84 $\pm$ 1.54 ms in the deep articular cartilage (B). The 3D fat saturated UTE Cones sequence shows high signal but low contrast for the ZCC (green arrows), with a $T_2^*$ value of 1.27 $\pm$ 0.41 ms (D).
Where $s$ is the signal intensity and $a$ is an unknown parameter.

**Results**

The clinical FSE and CPMG sequences show no signals in ZCC due to its short T2. The T2-weighted CPMG sequence showed T2 values of 35.63 ± 7.54 ms on average in the deep layer of articular cartilage. However, the used 3D UTE Cones sequence generated high-quality MR images that adequately sampled the signal decay pattern of ZCC. The ZCC can be better depicted in the subtracted image where a later echo image was subtracted from the first one. Based on the 3D UTE Cones sequence, the single-exponential fitting curve in ZCC was accurately obtained with R2 of 0.989 on average from 12 cadaveric and 10 volunteer knee joint [Figure 2].

Figure 3 shows 3D UTE Cones images with TEs=0.032/4.3 ms and the corresponding subtracted image [Figure 3C], which shows a high signal intensity of soft tissue calcification (arrows 1 and 2) and the ZCC (arrows 3 and 4). Single-component fitting of the multi-TE data suggested a short T2* of 2.2 to 4.1 ms for soft tissue calcification and 1 to 2 ms for the ZCC.[21]

For cadaveric knee joints, the ZCC has a short T2* ranging from 0.93 to 0.52 ms, with means ± standard deviation (SD) of 1.49 ± 0.66 ms, and with 95% confidence intervals (CI) of 1.20 to 1.78 ms. Error bars in Figure 4 represented the fitting standard errors of the mean (SEM) of T2* values in ZCC. For the volunteer knee joints, the short T2* ranges from 0.62 to 2.35 ms, with the mean ± SD of 2.09 ± 0.56 ms, and the 95% CI is 1.43–2.74 ms with SEM ranging from 0.38 to 0.93 in ZCC [Figure 5].

**Discussion**

**Technical feasibility of 3D multi-echo UTE Cones sequence for imaging whole knee joints on clinical trials**

Previously, the 2D UTE sequences have been employed to detect the T2* in ZCC using the DIR preparation pulse.[9] Compared with the 2D UTE sequences, the 3D UTE-Cones sequence has many advantages: first, the 3D UTE-Cones sequence using a short rectangular pulse for signal excitation is much less prone to eddy current artifacts compared with the 2D UTE sequences with a slice-selective half-pulse excitation[22]; second, the 3D UTE-Cones sequence allows 3D high-resolution volumetric imaging and quantification of ZCC in the whole knee. The 2D UTE sequence is only a single slice technique which may suffer out-of-slice long-T2 signal contamination and partial volume effects.[23] Third, much higher SNR can be derived from the used 3D UTE-Cones sequence because of its volumetric coverage over a single slice coverage. High SNR images were very important for accurate ZCC quantification due to the low proton density in ZCC.

In addition, compared with the conventional 3D radial k-space trajectory, the used 3D Cones k-space trajectory has more advantages including a better SNR performance and scan time reduction.[19,24] In our study, using 3D Cones k-space trajectory, we obtained high SNR ZCC imaging with the total scan times of 10.6 min for volunteer and 20 min for cadaveric knee joints. In this study, we performed T2* analysis of ZCC using 3D UTE-Cones ex vivo and in vivo human whole knee joints for the first time. Compared with conventional MR images [Figure 2], our preliminary results proved that the 3D UTE-Cones sequence together with a multi-echo acquisition strategy allow volumetric mapping of T2* in ZCC of whole knee joint of both samples and volunteer on a clinical 3T scanner, and could be further used to quantitatively evaluate the T2* relaxation time of the ZCC and soft tissue calcification, as shown in Figures 2 and 3. On the contrast, conventional MR sequences cannot be used for ZCC imaging with typical echo times of several milliseconds or longer.

**T2* values analysis using single components in ZCC**

Recent studies showed that UTE T2* mapping, with ultrashort TE of 0.032 ms or shorter, could detect short-T2 signal attenuation that depends on pulse spectral bandwidth (BW), Radiofrequency (RF) amplitude and the T2 values, that is not well captured by standard T2 mapping,[25] and also confirmed that T2* measurements could actually be explained by complex factors, including molecular (type II collagen and glycosaminoglycan) contents and microstructural levels (matrix architecture).[26] Moreover, Mort JS et al[11] have proved that the correlation of UTE-T2* values and polarized light microscopy (PLM) supports the hypothesis that T2* measurements may be more sensitive to collagen microstructure than to molecular contents.

In our study, data with a series of short echo times in a range from 0.032 to 8.8 ms, were acquired to measure T2* values. As results, we obtained that the T2* values ranged from 0.93 to 3.52 ms in ZCC of cadaveric knee joints that were lower than the results reported in preview studies. For example, Du et al[9] reported that on average the ZCC has a short T2* values ranging from 1.0 to 3.3 ms. There preview studies were consistent with the T2* values ranged from 0.93 to 3.52 ms in ZCC of volunteer knee joints. This could be explained the fact that the knee sample collected from the older cadaver, most of whom had suffered from OA.

On one hand, the decrease in T2* values in OA can be used to explain by the hypermineralized ZCC.[4] Ferguson et al[4] found that the ZCC was extremely and twice as hard as neighboring subchondral bone (SCB) in normal and OA human femoral head, and that the hypermineralized ZCC fragments may function as a grinding abrasive, accelerating wear rates whether attached to, or separated from the bony surface of the ZCC, and the hypermineralized ZCC could also alter loading patterns and thereby contribute to further destruction of the joint tissues. The hypermineralized ZCC in OA has also confirmed by Burr et al[27] using the anatomy and physiology study of ZCC. On the other hand, Du et al[9] studied DIR-UTE imaging and quantification of the ZCC and explained the net decrease in UTE-T2* value.
by a loss of water trapped within collagen fibrils (T2~4 ms) that may result in a relative increase of shorter T2 component intensities in the measured free induction decay (FID) curve.

However, our results showed that T2 values in ZCC are the same with previous results in normal ZCC, which can be explained that UTE-T2* was found to be more robust than standard T2 mapping in detecting.\cite{28}

Our study has several limitations. Firstly, no histology and PLM were performed in this study. In the future, we will investigate the correlation between quantitative 3D UTE-Cones findings and histopathological scores. Secondly, because of the different orientation of collagen in the different ZCC areas of the whole knee joints, T2* values were affected by the magic angle effect that was not investigated in this study. Measurement in different areas of whole knee joints would help to illustrate the angular

Figure 3: Knee joints of a 45-year-old male volunteer. High-resolution interleaved dual-echo 3D fat saturated UTE Cones MR imaging with TEs = 0.032/4.3 ms (A–B) and the corresponding subtraction image (C). High signal intensity can be seen from (C) for the soft tissue calcification (blue arrows 1 and 2) and the zone of calcified cartilage (ZCC) (arrows 3 and 4). T2* values of the above 4 regions of interests (ROIs) were obtained by the single-exponential fitting (D–G). The T2* values were 2.4, 4.1, 1.4 and 1.7 ms, respectively.
dependence of T2* values. Thirdly, although 3D UTE-Cones could give a high image resolution,[19] there still existed some partial volume effect. Finally, the total scan times of 10.6 min for volunteer and 20 min for cadaveric knee joints were still too long for patients on the clinic. Parallel imaging and compressed sensing reconstruction may be used to further reduce the total scan time.[29,30] More work will be needed to develop translational imaging.

Figure 4: Means of T2* values in the ZCC of 12 cadaveric knee samples. The smallest SEM was 0.53 ms, and the largest SEM was 1.21 ms among them. The gray trend line demonstrated that the data is stable enough to use 3-dimensional ultrashort echo time cones sequences projection imaging of the zone of calcified cartilage in cadaveric knee samples. SEM: Standard error of the mean; ZCC: Zone of calcified cartilage.

Figure 5: Means of T2* values in the ZCC of 10 volunteer knee. The smallest SEM was 0.38 ms, and the largest SEM was 0.93 ms among them. The gray trend line demonstrated that the data is stable enough to use 3-dimensional ultrashort echo time cones sequences projection imaging of the zone of calcified cartilage in volunteer knee joint. SEM: Standard error of the mean; ZCC: Zone of calcified cartilage.
of the ZCC in vivo. Further optimization of the imaging protocol, including thinner slices to minimize partial volume effects, more advanced reconstruction techniques and high performance localized coils, are required. Further exploration of UTE-T2* mapping as a noninvasive tool to detect early articular cartilage degeneration is warranted.\(^\text{[1]}\)

In conclusion, these preliminary results show that the high-resolution 3D UTE-Cones sequence can be used to directly image and quantification the ZCC in whole knee joints on a clinical 3T scanner. Direct imaging of ZCC in vivo may be particularly useful for diagnosis of articular cartilage degeneration and OA, as well as for monitoring treatment.

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Conflicts of interest

None.

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