Review Article

Detection of Repair of the Zone of Calcified Cartilage with Osteoarthritis through Mesenchymal Stem Cells by Ultrashort Echo Time Magnetic Resonance Imaging

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Abstract

Objective: Currently, magnetic resonance imaging (MRI) is the most commonly used imaging modality for observing the growth and development of mesenchymal stem cells (MSCs) after *in vivo* transplantation to treat osteoarthritis (OA). However, it is a challenge to accurately monitor the treatment effects of MSCs in the zone of calcified cartilage (ZCC) with OA. This is especially true in the physiological and biochemical views that are not accurately detected by MRI contrast agents. In contrast, ultrashort time echo (UTE) MRI has been shown to be sensitive to the presence of the ZCC, creating the potential for more effectively observing the repair of the ZCC in OA by MSCs. A special focus is given to the outlook of the use of UTE MRI to detect repair of the ZCC with OA through MSCs. The limitations of the current techniques for clinical applications and future directions are also discussed.

Data Sources: Using the combined keywords: "osteoarthritis", "mesenchymal stem cells", "calcified cartilage", and "magnetic resonance imaging", the PubMed/MEDLINE literature search was conducted up to June 1, 2017.

Study Selection: A total of 132 published articles were initially identified citations. Of the 132 articles, 48 articles were selected after further detailed review. This study referred to all the important English literature in full.

Results: In contrast, UTE MRI has been shown to be sensitive to the presence of the ZCC, creating the potential for more effectively observing the repair of the ZCC in OA by MSCs.

Conclusions: The current studies showed that the ZCC could be described in terms of its histomorphology and biochemistry by UTE MRI. We prospected that UTE MRI has been shown the potential for more effectively observing the repair of the ZCC in OA by MSCs *in vivo*.

Key words: Magnetic Resonance Imaging; Mesenchymal Stem Cells; Osteoarthritis; Ultrashort Echo Time; Zone of Calcified Cartilage

Introduction

The initial changes in osteoarthritis (OA) always occur in the noncalcified part of the articular cartilage (AC), followed by gradual damage to the zone of calcified cartilage (ZCC); however, OA sometimes starts ZCC that could be the subtypes of OA.[1,2] Therefore, rebuilding the ZCC is an essential strategy in the treatment of OA. The use of bone marrow mesenchymal stem cells (MSCs) to repair the ZCC in some studies represents a new milestone in the treatment of degenerative OA.[3-6] However, the ZCC has a short T2, and its signal decays quickly after radiofrequency excitation; as a result, little or no signal is shown in the ZCC with conventional clinical magnetic resonance imaging (MRI) techniques, such as gradient recalled echo, fast spin echo, short-TI inversion recovery, and so on. Together with positive and negative contrast agents such as superparamagnetic iron oxide (SPIO), delayed gadolinium-enhanced MRI

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.4103/0366-6999.224725

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Received: 16-10-2017 **Edited by:** Xin Chen

How to cite this article: Zhou Q, Li SL, Ma YJ, de Tal V, Li W, Zhao YH. Detection of Repair of the Zone of Calcified Cartilage with Osteoarthritis through Mesenchymal Stem Cells by Ultrashort Echo Time Magnetic Resonance Imaging. Chin Med J 2018;131:1092-8.

of cartilage (dGEMRIC), SPIO nanoparticles (SPIONs), ultrasmall SPIONs (USPIONs), magnetodendrimers, and so on. MR tracking of contrast agents labeled cells might lead to detrimental effects on tissues *in vivo*. Therefore, the ZCC's degradation and biochemical information cannot be observed, and quantitative parameters of the ZCC cannot yet be measured by MRI during the treatment of OA by MSCs.

In recent years, the characteristics of the ZCC can be better visualized with ultrashort time echo (UTE) than with conventional MRI.[7] With the efficient suppression of the long T2 tissues, such as the superficial layers of AC and bone marrow fat, a linear high signal of the ZCC can be clearly displayed [Figure 1]. Therefore, UTE MRI may be able to evaluate the response of the ZCC to MSC treatment of OA without the use of ionizing radiation. This review mainly addressed the feasibility of using UTE MRI to monitor the physiological and biochemical changes in the OA-afflicted ZCC during MSCs treatment *in vivo*.

Repair of the Zone of Calcified Cartilage with Osteoarthritis through Mesenchymal Stem Cells

Structural and pathological changes of the zone of calcified cartilage with osteoarthritis

AC can be divided into four regions from its surface to its deepest layers: (1) the surface area, (2) the cartilage transition zone, (3) the cartilage radial zone, and (4) the ZCC.[8] The ZCC is a highly modified mineralized region and accounts for about 8%, that seems too thick, of the total thickness of the AC. Water accounts for about 65% of the ZCC,[9] which is composed of bound water combined to proteoglycans (PG) and collagen (T2*, hundreds of μs to several milliseconds) and bulk water residing in the macromolecular matrix (T_2*, tens of milliseconds). These present in most joint tissues including AC.[10] With the onset of OA, the macromolecular structure of PG and collagens are damaged, collagen fibers are swollen, the tension of collagen fibers is decreased, so that bound water is reduced, and the free water is increased.[11]

Micro-cracks of the ZCC occur under repeated stress due to fatigue, so the number of micro-cracks is much higher in weight-bearing areas than in nonweight-bearing areas. In the early stages of OA, the ZCC and the growth plate are remodeled, and the cartilage thins. The cells in the ZCC express a hypertrophic chondrocyte phenotype and secrete type X collagen, and then cartilage matrix mineralization occurs. As a result, the tide line moves forward, and subchondral bone abnormalities occur, and this causes degeneration of cartilage.[12]

Interactions of cartilage and subchondral bone mediated by the zone of calcified cartilage with osteoarthritis

The superficial layers of AC and bone are connected by the ZCC, which allows redistribution of forces at the interface and enhances mechanical integration.[13] Therefore, ZCC defines the structure of an important interface between cartilage and subchondral bone and plays a crucial role in the pathogenesis of OA.[14] The chondrocyte phenotype osteoblasts in hardened subchondral bone may result in hypertrophic chondrocytes. The thickness of the ZCC is maintained by a balance between progression of the tidemark movement into the nonmineralized cartilage and the ossification by vascular invasion and bone repairing. Osteoblasts of osteosclerotic subchondral bone synthesize many pro-inflammatory cytokines that affect cartilage cells in the noncalcified cartilage through the micro-cracks in the ZCC. Therefore, reconstruction of the ZCC in osteochondral conjunction has become one of the hot research topics in the field of interface tissue engineering.[14]

Monitoring the Process of the Zone of Calcified Cartilage Improvement with Osteoarthritis *In vivo*

Physiological and biochemical characteristics of recovery of the zone of calcified cartilage with osteoarthritis *in vivo*

Currently, when observing the improvement of AC by MSCs *in vivo*, several criteria need to be considered, including levels of bone morphogenetic protein, transforming growth factor-β, fibroblast growth factors, insulin growth factor-1, hedgehog, wingless (Wnt) proteins, and so on.[15] Through immunophenotype, some markers used to identify MSCs are the positive protein enzymes for STRO-1, CD73, CD146, CD105, CD106, and CD166; other markers are the negative protein enzymes for CD11b, CD45, CD34, CD31, and CD117.[16,17] Information about the formation, growth, and degeneration of the AC is mostly obtained through microscopy

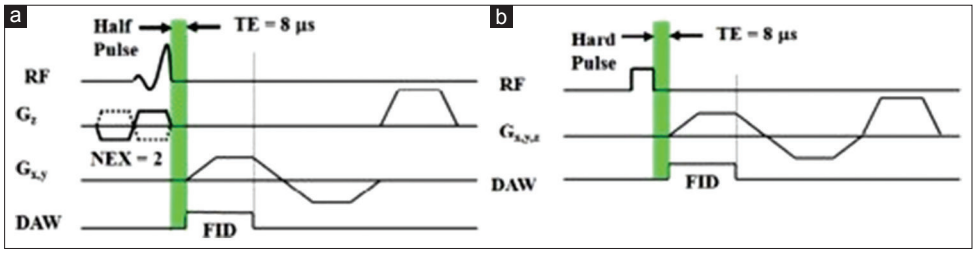

Figure 1: Diagram of ultrashort time echo pulse sequence. (a) Two-dimensional semi-RF pulse combined with optimized selection gradient layer optimization of opposite polarity to motivate one layer, using two-dimensional radial motivation trajectories-filled k-space. (b) After excitation of a short rectangular hard pulse, three-dimensional radial track k-space is used. RF: Radiofrequency.

by observing the surface roughness and thickness, collagen level, and the content and percentage of the hydroxyapatite of the AC.[18] With MSCs in the joint, the AC is thickened, and MSCs may be digested by hydrolase, and the content of collagen is also significantly increased. The expression of chondrocyte matrix metallopeptidase (MMP)-1 and MMP-3 is significantly increased, and the permeability level is also increased in OA.[19] Ultrastructural recovery of the AC is most likely to be reflected through the decrease in bulk water and increase in collagen inside the damaged cartilage.

However, there is currently little observation of the ZCC with OA *in vivo*. Only one case was reported how the ZCC with rheumatoid arthritis (RA) was selectively destroyed by Zschäbitz,[20] in which non-ZCC was preserved integrally, and no treatment was described in the destroyed ZCC. This method, used to detect the process of the destroyed ZCC, was not only very complicated but also wasted a lot of experimental resources. Hence, it is necessary to develop a new technique detecting how the ZCC interface is destroyed with OA, and how the special physiological and biochemical function of the destroyed ZCC is recovered *in vivo*.

Monitoring the recovery of osteoarthritis through magnetic resonance imaging

MRI is a noninvasive technique used to evaluate the progress of degeneration in OA.[21] MRI can show changes that accompany OA in the molecular, cellular, and subcellular levels of the tissue. MRI can quantitatively demonstrate changes in the physiological and pathological processes that occur during OA. Hence, it can also elucidate the biological mechanisms in the treatment of OA.[18]

MRI technologies to detect physiological changes of AC have been developed in recent years, such as T2*-mapping, spin–lattice relaxation in the rotating frame (T1rho) imaging, Na imaging, delayed gadolinium-enhanced MR (dGEMR) imaging, T1 mapping with contrast (dGEMRIC), magnetization transfer (MT), and diffusion-weighted imaging (DWI).[22] T2* mapping can provide a tool for monitoring changes in water and collagen. T1rho imaging is predominantly sensitive to changes of PG in early-stage OA.[23] The Na imaging method is considered to be very sensitive to measure the content of PG in AC;[24] therefore, it can be used to detect physiological and biochemical changes in cartilage. The dGEMRI has high resolution and sensitivity when estimating joint cartilage glycosaminoglycan content. MT is a valuable tool to compare contrast agents between cartilage and fluid in the joint. DWI is sensitive to the change in postoperative imaging.[25] These new techniques can be used to measure IB, including T2* value, T1rho value, T1Gd index, and others. The IB can indirectly indicate the content of glycosaminoglycans, PG and collagen, the collagen fiber anisotropy, water contents, and the low-frequency flow between water and large molecules.[26] For example, Mosher *et al*.[27] reported that morphometric biomarkers of MRI could be used to evaluate cartilage changes, with intraclass correlation coefficients, ranged from 0.61 to 0.98, and root mean square (RMS) coefficients of variations (CVs) ranged from 4% to 14%.

Tracking and quantifying the treatment of osteoarthritis using mesenchymal stem cells by magnetic resonance imaging *in vivo*

In recent years, conventional MR techniques together with positive and negative contrast agents after stem cell transplantation have been used to observe the growth and development of the stem cells transplanted *in vivo*. Both cellular total iron load and the localization of particles of iron oxide through SPIO labeling have been used to reveal a decrease of migration capacity and colony-forming ability. Research is booming on the properties of MRI and the properties of MSC labeling methods. For example, a recent study by Roeder *et al*.[28] showed that using SPIO, MRI could trace MSCs long-term *in vitro* and that low concentrations (12.5–25.0 μg Fe/ml) of SPIO on TGF-beta1 driven chondrogenesis optimized both chondrogenesis and MRI labeling. In another example, Addicott *et al*.[29] introduced Molday ION Rhodamine B (MIRB) as a new SPIO contrast agent designed for cell labeling that was easily internalized by nonphagocytic cells. Average MIRB per MSC ranged from 0.7 pg Fe for a labeling MIRB concentration of 5 μg Fe/ml. The Fe/MSC ratio asymptotically approached a value of 20–25 pg/cell as labeling concentration was increased to 100 μg Fe/ml. These investigations are just two of many that have laid the foundation for using contrast agents, including SPIO, SPIONs, USPIONs, magnetodendrimers, and so on, with MRI to detect the AC *in vivo*.[30,31]

However, the current use of MRI does not accurately show how the ZCC with OA evolves physiologically and biochemically;[32] in particular, the recovery of the ZCC using MSCs has so far not been detected by MRI.

Monitoring the zone of calcified cartilage's progression with osteoarthritis by ultrashort time echo magnetic resonance imaging

During the past decade, UTE MRI has made it possible to detect the short-T2 signals of the ZCC,[33,34] and UTE spectral imaging has shown that T2* values of the ZCC are 1–2 ms, and treatment effects of MSCs on cartilage has been evaluated by UTE MRI.[35] Using UTE MRI, previous studies showed the decrease in T2* values in ZCC explained by the hypermineralized in OA.[36] Excellent image contrast is achieved for the deep layers of cartilage in the subtracted image, and the deep layers of cartilage are shown as a high signal line above the subchondral bone (arrows).[37]

The water and macromolecular environment determine the T1 relaxation time of tissues,[38] so T1 is markedly decreased in the ZCC as compared to the superficial cartilage in knees.[39] Furthermore, increased T1 is associated with cartilage degeneration.[25] In the ZCC, the short T1 component accounts for about 70% of the signal, with another 30% from the adjacent long T1 component likely to be due to the partial volume effect.[40]

T1rho has been proposed as an attractive alternative to probe biochemical change in cartilage. It reflects the slow interactions between motion-restricted water molecules and their local macromolecular environment, and has a high sensitivity to PG loss in bovine cartilage samples as well as to the presence of OA in patients.[41] Dual inversion recovery UTE (DIR-UTE) T1rho images show the ZCC of a patella sample with excellent contrast which is hardly visible with conventional UTE-T1rho imaging. Exponential fitting of the DIR-UTE image shows a short T1rho of 4.61 ± 0.07 ms.[13]

Recently, studies have demonstrated that the T2* of deep cartilage is sensitive to matrix degeneration.[25] Mono-exponential fitting shows the T2* of the ZCC to be 1.79 ± 0.2 ms, confirming the need to have the appropriate dynamic range and recommendation for TE values utilized for UTE of the ZCC.[13] UTE imaging together with a bi-component T2* analysis has the potential to quantify T_2*, including the short and long T_2 fractions of water components in tissues *in vivo*. For AC, the UTE T2* decay curves show two distinct components with a monotonic increase in short T2* fraction from 15.1% for the superficial zone, to 17.1% for the middle zone and 21.6% in the deep zone. This may be partly explained by the gradual increase in the concentration of collagen. Furthermore, the collagen fibrils near the articular surface are of a much smaller diameter and more closely packed.[42] Williams *et al*.[43] showed that UTE-T2* mapping could quantitatively evaluate bulk water, bound water and organic matrix of the AC, which could indirectly show the content of collagen and fiber anisotropy. Pauli *et al*.[44] reported that Carr-Purcell-Meiboom-Gill measured T2 values of normal patellar cartilage specimens varied from 30.7 ms to 79.3 ms, and the short T2* water fraction ranged from 18.8% to 20.7%.

New Techniques to Image and Quantify the Zone of Calcified Cartilage

In two-dimensional UTE MRI, the half pulse excitation is sensitive to gradient imperfections, such as eddy current distortions, that cause errors in the slice profile. Furthermore, MRI of the fine structure of the ZCC is susceptible to the partial volume effect. Using short and hard pulse excitation and three-dimensional radial collection, UTE imaging can minimize the influence of the partial volume effect [Figure 1b].[32]

Three-dimensional UTE-T2* mapping on AC is achieved at 3T MR, and the accuracy error of the UTE-T2* values is below 1.2 ms, or 8 RMS average (RMSA) CV, in regions of interest (ROIs) in the loading areas of AC of asymptomatic subjects. Williams *et al*.[43] examined 11 cases of medial tibial condyle and plateau in weight-bearing conditions using enhanced three-dimensional UTE-T2* MRI. They found when they repeated the T2* value measurements, the changes in the RMSA coefficient in the deep layers of AC were 16% for the medial tibia condyle and 13% for the plateau, and the absolute errors were respectively 1.5ms and 2.1ms. Using UTE MRI sequences, Pauli *et al*.[44] observed that water with short T2* correlated significantly with histopathological grading (Mankin classification) using the method of polarized light microscopy (PLM; Vaudey classification).

To overcome some limitation, such as high SAR, low resolution, and partial volume effects due to two-dimensional imaging, three-dimensional fat saturated UTE cones sequences, which is a novel technique to analyze the contents of the ZCC, were implemented on a 3T Signa TwinSpeed scanner (General Electric Healthcare Technologies, Milwaukee, WI, USA) by us to develop high contrast imaging of the ZCC. The following imaging parameters were used: a field of view (FOV) of 12 cm × 12 cm × 8 cm, a slice thickness of 2 mm, a reconstruction matrix of 320 × 320 × 40, a sampling BW of 125 kHz, 144 min scan time, and 15° flip angles. To measure T2*, three-dimensional fat saturation UTE cones images were acquired at a series of TEs of 0.1, 0.2, 0.4, 0.6, 0.9, 2.2, and 6.6 ms [Figure 2a–2g]. The total scan time was about 20 min for T2* quantification. T2* of the ZCC was measured with three-dimensional fat saturation UTE cones acquisitions at progressively increasing TEs.

T2* values were obtained using a Levenberge–Marquardt fitting algorithm written in Matlab (The Mathworks, Natick, MA, USA). The ZCC of the cadaveric knee joint can be best depicted in a subtraction image [Figure 2h], where a later echo image was subtracted from the first one. The UTE images were assessed using single-component analysis at ROIs drawn in the ZCC [Figure 2i]. The ZCC of the cadaver has a short T2* ranging from 0.7 to 3.3 ms [1.45 ± 0.66 ms; Figure 2j]. We concluded that three dimensional fat saturation UTE cones MRI could be used for quantitative evaluation of the ZCC's T2* relaxation times.

UTE MRI techniques have high spatial resolution, broad coverage, and high contrast to show short T2 species.[45] The quantitative analysis of the ZCC by UTE MRI could provide a noninvasive method for detecting the contents and collagen orientation in the ZCC. Brossmann *et al*.[46] reported that UTE (TE = 150 μs) MRI could display early changes in the ZCC with OA, and its sensitivity rate achieves 100%. Therefore, these measurements are useful for understanding the changes of this tissue component in OA, and UTE techniques can potentially be used to monitor the ZCC's progression in OA.

Outlook

Due to rapid improvements in MRI, the technology of UTE MRI has become sensitive to the presence of the ZCC.[36] Using UTE MRI, the quantities of total water, bound water, bulk water, and their fractions can be accurately measured during the quantitative assessment of the ZCC.[25] UTE MRI may reflect the correlation between IB and structure, so it may show the physiology and biochemistry of the ZCC *in vivo*, and quantitative UTE MRI techniques, for example, T2 mapping, T1rho, dGEMR, and DWI, may noninvasively

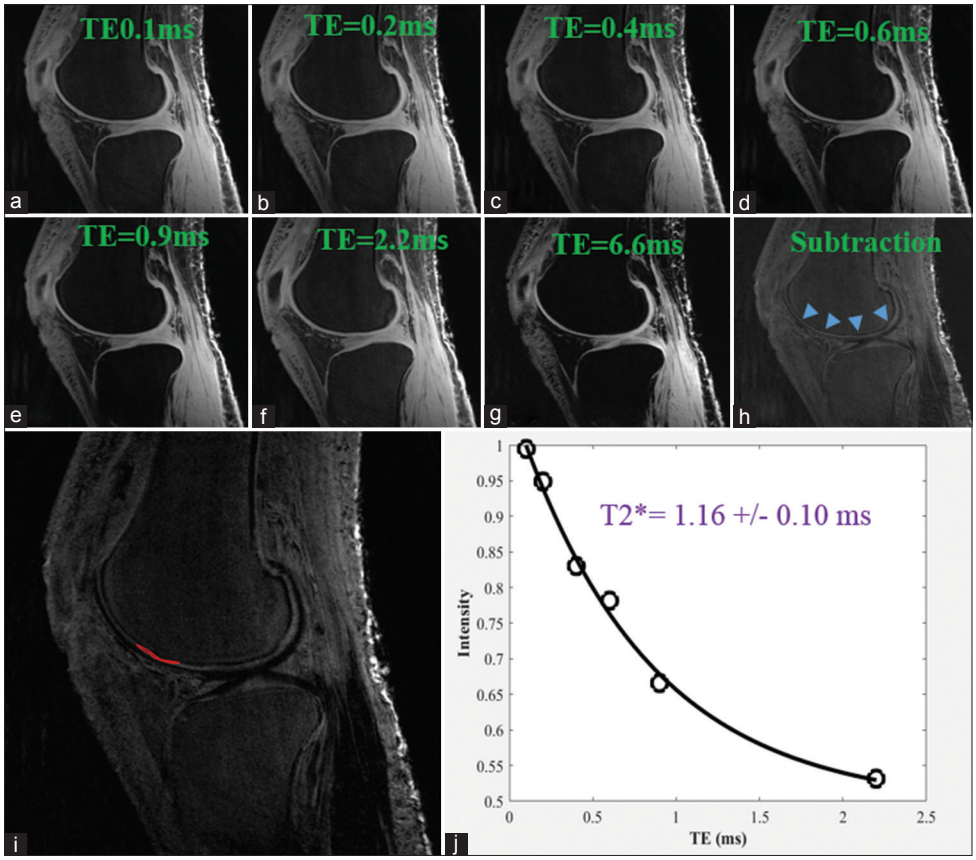

Figure 2: Dual echo three-dimensional fat saturation UTE cones MRI of ZCC from a 40-year-old cadaveric knee joint specimen. UTE images were acquired at a series of TEs of 0.1 ms (a), 0.2 ms (b), 0.4 ms (c), 0.6 ms (d), 0.9 ms (e), 2.2 ms (f), and 6.6 ms (g). The eighth image was produced by a subtraction of the seventh one from the first one, wherein the visualization of the ZCC of knee cartilage was improved (arrows) (h). A single-component exponential fitting curve (j) showed a short T2* of 1.16 ± 0.10 ms at ROIs in the ZCC (i), and there was loss of signal with increasing TE. ZCC: Zone of calcified cartilage; UTE: Ultrashort time echo; MRI: Magnetic resonance imaging; ROIs: Regions of interest; TE: Time echo.

provide information about tissue biochemistry of the progress of the ZCC with OA *in vivo*. IB of the ZCC, such as T1, T1rho, T2* values, and so on, can be measured to indirectly assess the collagen content in the organic matrix, the structure of collagen fibers, mineralization, hardness, permeability, and other information *in vivo*.

Although a variety of UTE MRI methods have been used to qualitatively and quantitatively evaluate the ZCC, no research reports were found on the quantitative assessment of the process of the repair of the ZCC by MSCs *in vivo*. Changes in bound water, bulk water, and their fractions inside the ZCC may provide a physiological basis for UTE MRI to detect changes in the OA-affiliated ZCC during repair by MSCs *in vivo*. In summary, UTE MRI could become the standard method for monitoring the progress of treatment of the ZCC with OA using MSCs *in vivo*. This would eliminate the need to sacrifice animals and would rescue the inability of conventional MRI methods to quantitatively assess the ZCC with OA. More studies on the imaging of ZCC should be performed to establish new validation and verification against a standard, likely requiring preclinical animal models for validation.

However, some obstacles remain when using UTE MRI to detect the ZCC. For example, changes in cartilage T2 relaxation time are nonspecific and influenced by multiple factors, including hydration, macromolecular content, tissue anisotropy, and others. Changes in cartilage T2 relaxation time also occur within the range of variability of results produced by different hardware settings, making comparison across different experiments problematic. Meanwhile, *in vivo* cartilage imaging is time- and subject-constrained, resulting in decreased spatial resolution, reduced SNR, and motion artifacts. More work will be needed to develop translational imaging of the ZCC *in vivo*, for example, reduction of imaging time, decreasing partial volume artifacts, improve the resoluti., With these unresolved issues, necessary improvements include optimization of UTE MR techniques, further development of reconstruction techniques, and improved performance of localized coils. The ranges of T1, T1rho, and T2* of the ZCC should be further investigated, and the correlation between the IB on UTE MRI and histopathological scores on PLM should be clarified.

Conclusion

Compared to all other methods to treat OA, MSCs have the advantage of repairing both the structure and the biological function of the ZCC with OA. However, in current studies,

many animals must be sacrificed to detect physiological changes of the ZCC after MSCs transplantation. This process is not only complex but is an inefficient use of time and other resources. Currently, no MRI or other modalities are sufficient in evaluating the ZCC after stem cells are transplanted *in vivo*. Shortcomings of current methods include low sensitivity, radioisotope decay, cell toxicity owing to marked stem cells, and possible toxicity to cartilage cells because of genetic modification.[47] UTE MRI of the ZCC is a novel qualitative and quantitative MRI technique with the potential to visualize ZCC characteristics. With UTE MRI, the linear high signal of the ZCC can be clearly displayed, and T1, T1 rho, and T2*value of the ZCC can be reliably measured. The technology of UTE MRI offers promise for the structure, physiology and biochemistry of the ZCC with OA, and could provide an objective evaluation of the regenerative process of the ZCC *in vivo*. Therefore, UTE MRI can potentially be used to evaluate the reaction of the OA-afflicted ZCC to treatment with MSCs *in vivo*. However, using UTE MRI to qualitatively and quantitatively assess changes of the ZCC with OA after MSCs transplantation is still challenging. Issues include hardware and software limitations, software availability, and a lack of validation studies.[48] Further studies on UTE MRI are needed to determine how it can effectively be used to monitor the progress of treatment of the ZCC with OA using MSCs *in vivo*.

Financial support and sponsorship

This work was supported in part by grants from the Science and Technology Plan Projects of Guangdong provincial, China (No. 2014A020211018 and No. 2014A020212399) and the Tianhe District Science and Technology Project of Guangzhou city, China (No. 201704KW026) .

Conflicts of interest

There are no conflicts of interest.

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基于超短回声时间磁共振成像检测间充质干细胞修复骨关节炎软骨钙化区的展望

摘要

目的: 磁共振 成像(MRI)是观察骨髓间充质干细胞体内移植治疗骨关节炎 (OA) 最常用的影像学手段。 然而, 准确监测骨髓间充质干细胞对OA软骨钙化层 (ZCC) 的疗效依然存在挑战。 采用核磁共振标记物介入无法准确检测ZCC的生理和生化变化。 相反，有研究表明超短回波时间 (UTE) 磁共振成像能敏感地发现ZCC的变化, 从而有可能更有效地观察MSCs对ZCC修复。 因此, 本文章重点关注UTE MRI观察骨髓间充质干细胞修复ZCC的应用前景, 同时讨论当前技术在临床应用中的局限性和未来的发展方向。
数据来源: 采用关键词：“骨关节炎”， “间充质干细胞”， “钙化层”和“磁共振成像”，利用PubMed/MEDLINE检索功能进行高级搜索,检索时间截止到2017年6月1日。
选择文献: 初步共检索到132篇已发表的相关文献。 经过进一步的详细审查, 本综述分析了48篇英文文献。
结果: 研究表明UTE MRI检测ZCC具有较高的敏感性和较大的潜力, 可有效地观察MSCs在OA的ZCC修复过程。
结论: 目前的研究表明, 可以通过UTE MRI描述ZCC在组织形态学和生物化学方面的变化。 UTE MRI具有在活体内观察MSCs修复OA ZCC的潜力。