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# 关节软骨显微成像技术

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**摘要**:对关节软骨组织成像研究中磁共振成像(MRI和μMRI)、偏振光显微术(PLM)、傅里叶变换红外成像(FTIRI)及计算机断层扫描成像(CT)等技术方法进行了综述,指出每一种技术方法利用其自身技术原理均能描述组织退化复杂机制的一个方面,但多学科交叉法被认为是最好的技术手段.

**关键词**:关节软骨退化;显微成像;多学科交叉;核磁共振;偏振光显微镜;傅里叶变换红外成像
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## Articular cartilage by microscopic imaging

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**Abstract**: The multidisciplinary research techniques in the study of articular cartilage tissue imaging were reviewed. The magnetic resonance imaging (MRI), microscopic MRI (μMRI), polarized light microscopy (PLM), Fourier-transform infrared imaging (FTIRI) and computer tomography (CT) were emphatically introduced. It was pointed out that each technique could probe an aspect of the tissue degradation complex mechanism with its own scientific principles. But in the current study of biomedical problems, the multidisciplinary approach is the best.

**Key words**: articular cartilage degeneration; microscopic imaging; multidisciplinary approach; MRI; PLM; FTIRI

## 0 Introduction

The gradual degradation of articular cartilage is a hallmark of osteoarthritis (OA), which is the number one cause of disability in the U. S. population[1]. OA progresses very slowly, often spanning over ten or twenty years. Since cartilage is avascular and aneural, early degradation remains silent (i. e., undetected). Although OA always has the same end-stage symptoms (i. e., losses in joint function), the disease can have different early-stage characteristics, each being the consequence of a different initiation event (or risk factor), such as trauma, obesity, or instability. Due to these complexities, an accurate diagnosis of early OA in humans, at a time when the joint is still structurally sound but its load-bearing ability is compromised, remains elusive in current clinical practice. Since one cannot initiate diseases in humans, nor test some potential disease-modification drugs on humans before their effectiveness is demonstrated, the use of animal models in OA research serves an essential purpose[2-3].

Articular cartilage is a thin layer of load-bearing tissue covering the ends of the bones in a diarthrodial joint (Fig. 1). Cartilage is composed of a small amount of cells (chondrocytes) and an extensive extracellular

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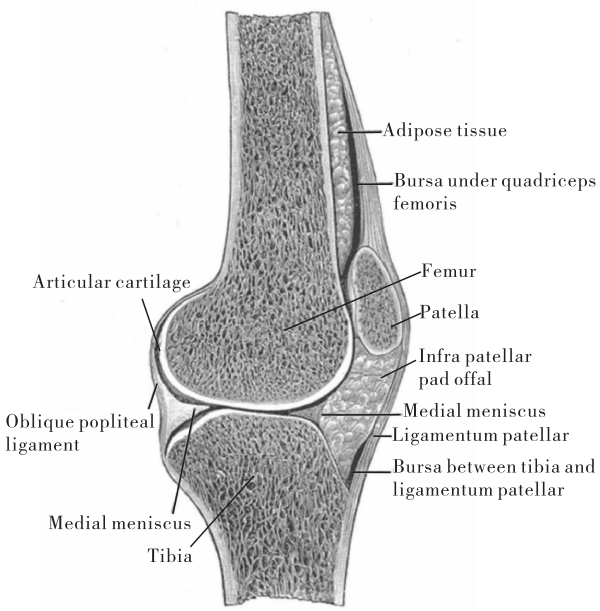
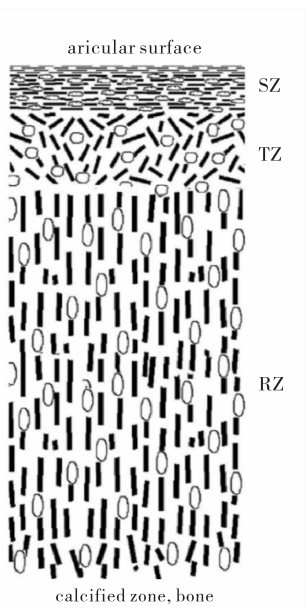
matrix, which has three major constituents: water, collagen fibers (mostly type II), and proteoglycan (PG, a protein with many covalently attached chains of glycosaminoglycans (GAG)). A unique structural feature of articular cartilage is its depth-dependent characteristics across its thin thickness, which is signified by a nominal 90° orientation change between the surface and the deep tissue (Fig. 1b)). Consequently, a thin layer of articular cartilage is commonly sub-divided in histology, based on the local fibril orientation, into three sequential zones depth-wise: the superficial zone (SZ), where the fibers run parallel with the articular surface; the transitional zone (TZ), where the fibers are randomly oriented; and the radial zone (RZ), where the fibers are perpendicular to the surface. Nearly all biological and molecular properties of cartilage consequently bear some type of depth-dependency with regard to its thin thickness[4-12]. Even at the proteomic level, a recent study has found, for the first time, the depth-dependent zonal distribution of a broad range of cartilage ECM proteins[13].

Since early lesions tend to be small and localized, any early detection tool must be non-invasive, sensitive to molecule-specific changes in the tissue, and able to resolve small lesions. Molecular imaging at high resolutions meets these requirements. During the last twenty-one years, we have been studying the degradation of articular cartilage extensively in our laboratory at Oakland University. Initially, we used μMRI, which shares the same physics principles and engineering architectures as in clinical MRI scanners. In the later 1990s, we started to incorporate polarized light microscopy (PLM) into our work, because PLM is the gold standard in histology and capable of visualizing the fibril organization in cartilage, which modulates the μMRI signal. As our projects progressed, we aimed to image the early lesions in cartilage as directly as possible, at high resolutions. For this reason, we started to use Fourier-transform infrared imaging (FTIRI) in our work, since FTIRI is sensitive to the chemical bonds in the tissue. One of the latest additions to our research tools is microscopic computer tomography (μCT), which enables the investigation of the cartilage-bone interface. In addition to these high-resolution imaging tools, we also employ several biomechanical and biochemical methods—each measures a unique aspect of the tissue's bulk properties and can be correlated with the spatially resolved changes in imaging. This multidisciplinary research approach in our cartilage research is recognition that many of today's biomedical problems are best




a) A schematic of the articular capsule of the knee joint




b) A schematic of the depth-dependent nature of articular cartilage

Fig. 1 Articular cartilage

addressed using multi-disciplinary techniques; each method has its own scientific merit[14]. This article provides a brief summary of our multi-disciplinary research in cartilage and osteoarthritis.

# 1 Microscopic imaging

## 1.1 Magnetic resonance imaging (MRI) and microscopic MRI (μMRI)

NMR imaging (MRI) is based on a linear proportionality between the precessional frequency of the nuclei (e. g., protons in water molecules) and the magnitude of the external magnetic field in which the nuclei are immersed[15-16]. By making the magnetic field in the sample space deliberately non-uniform, the spatial positions of the nuclei can be encoded by the variations of their precessional frequency. A set of three orthogonal gradients will enable the precise localization of any nucleus in a three dimensional space. When the resolution of MRI is finer than 100 μm, the smallest size that can be recognized by a naked eye, MRI is termed NMR microscopy or μMRI[17-19]. Since μMRI and clinical MRI share the same physics principles and engineering architectures, μMRI offers a direct translational bridge between invasive imaging (e. g., light microscopy and electron microscopy, which have higher resolution) and non-invasive MRI of humans.

The unique value of MRI is the combination of its true non-invasiveness and its excellent sensitivity to a number of molecular-level characteristics in the specimen. These characteristics may include magnetic susceptibility variation due to the hetero-structure of a sample, relaxation processes of the nuclei, chemical shift variations, and molecular motions (translational flow and Brownian motion)[19]. In MRI studies of articular cartilage, measurements of relaxation times ($T_1$, $T_2$, $T_{1\rho}$) have particular importance. For example, $T_2$ (spin-spin relaxation time) in articular cartilage has a depth-dependent anisotropy (Fig. 2c))[20-21], which is caused by the nuclear dipolar interaction of the protons under the influence of the collagen architectures in cartilage[22-23]. This $T_2$ anisotropy, which follows the geometric factor $(3\cos^2\theta - 1)$ in dipolar Hamiltonian, is the origin of the magic angle effect in clinical MRI of cartilage[22-30]. In contrast to the strongly anisotropic $T_2$ in cartilage, $T_1$ (spin-lattice relaxation time) in native cartilage is isotropic[23]. These isotropic $T_1$ and anisotropic $T_2$ times precisely reflect the slow molecular motion in cartilage due to the highly constrained and swollen PG in the collagen matrix (i. e., there is a large amount of proteoglycan macromolecules present in cartilage). With the use of the gadolinium-based contrast agent in MRI, we can also image the PG concentrations in cartilage quantitatively (Fig. 2d))[31], which provides a direct measure of the load-bearing ability of cartilage.

Since articular cartilage is a load-bearing tissue, an external loading should be able to change the fibril architecture and hence the relaxation characteristics in cartilage[32-33]. A diseased cartilage becomes easier to deform due to the reduction of its negatively charged proteoglycans. This implies that using static loading during microscopic imaging can become, in every sense, a functional study of the tissue's structures and properties. Imaging a cartilage block while it is being loaded in μMRI is analogous to imaging a joint in a human while a force or weight is applied to the person in a clinical MRI scanner-both rely on the articular cartilage to act its primary role as a load-bearing medium[34]. Detailed knowledge of the load-modified relaxation anisotropy in cartilage could provide a better understanding of structural modifications to the functional adaptability of cartilage, which could facilitate the monitoring of tissue degradation more accurately and provide critical information toward the understanding, and ultimately prevention, of arthritic diseases[35-40].

## 1.2 Polarized light microscopy (PLM)

Light microscopy is the gold standard in biology and histology labs, due to its low cost and high resolution (up to a fraction of one micron). Light in non-quantum mechanical physics can be treated as a transverse electromagnetic wave, which enters a specimen with a certain amplitude and phase. A characteristic of an anisotropic material is its intrinsic birefringence, i. e., an anisotropic material will have (at least) two

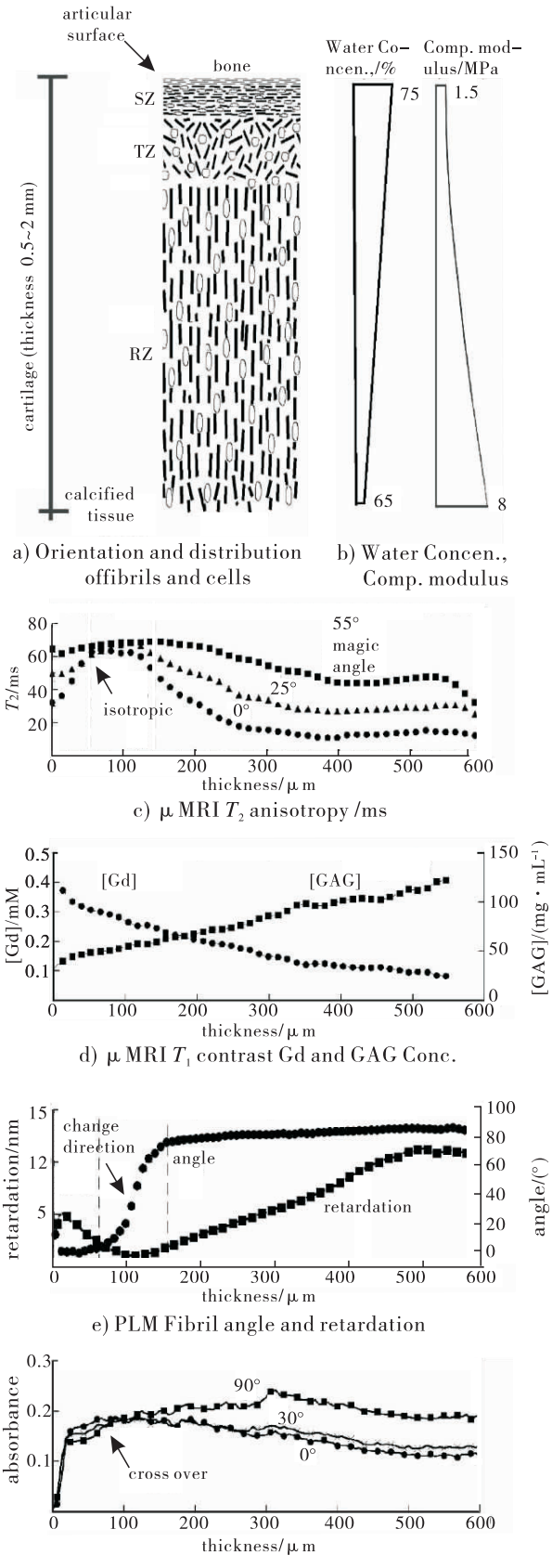



Fig. 2　Multidisciplinary correlation of the depth-dependent characteristics of articular cartilage

different indices of refraction, which result in two possible speeds of light transmission in the specimen. In articular cartilage, the polarized light interacts with the valence electrons of the collagen fibrils and is transmitted in two orthogonal axes. The difference of two indices of refraction results in the intrinsic birefringence, which is dependent on the angle between the axis of linear polarization and the axis of the fibrils[41-42]. The use of polarized light in light microscopy[43] enables the construction of two quantitative parameter images of the specimen: angle (in units of degrees) and retardation (in units of nm)[44-46].

The value of the angle in PLM represents the averaged orientations of all collagen fibrils within each image voxel (volume of element in imaging), which has a nominal 90° difference in the orientations between the superficial zone and the radial zone in articular cartilage (Fig. 2e))[44]. The value of the retardation characterizes the averaged organization of all collagen fibrils within each voxel (Fig. 2e)). A voxel of uniformly oriented fibrils would give the highest retardation value while randomly oriented fibrils would result in the smallest retardation value. In fact, both angle and retardation measurements can be used to model the averaged behaviour of molecules in cartilage using a generalized ellipse model[14]. Our work shows that the pixel-by-pixel mapping of angle and retardation allows investigations into the disrupted collagen morphology due to early onset lesions[35] and deformation of loaded articular cartilage[47-48]. A quantitative correlation study between μMRI and PLM successfully showed a statistically significant agreement between the structural zones in μMR images and in PLM images[44]. Since a disruption of the collagen morphology provides a clear signal to the onset of the cartilage degradation[49-51], PLM has been used extensively in recent years in cartilage and OA research[26, 35, 47-48, 52-54].

### 1.3　Fourier-transform infrared imaging (FTIRI)

FTIRI has been used extensively in cartilage studies during the last decade, due to its potential to provide quantitative and spatially resolved information about the chemical composition of the tissue[48, 55-62].

Infrared (IR) spectroscopy is based on the vibrational motions of the dipole moment of the molecule, which absorbs IR light when their vibrational frequency is equal to the frequency of incident radiation. In the mid-infrared region (4 000 ~ 400 $cm^{-1}$), the infrared spectrum of cartilage contains the major absorption of several biomedically important dipoles, such as amide I (C═O stretching), amide II (C—N stretching and N—H bending), amide III (N—H and C—C vibrations), and proteoglycan via sugar (C—C ring vibrations). FTIRI, which combines a classical Fourier-transform infrared spectrometer and an IR microscope that has a scanning stage, is a spectroscopic imaging technique that scans a 2D tissue section and produces a 3D spectroscopic imaging data cube, which contains both spatial ($X$ and $Y$ in microns) and chemical ($Z$ in wavenumbers) information. At any particular frequency or range of frequencies, one can construct a 2D spatial image of the particular chemical bonds, which allows the visualization of those chemicals in the specimen as a 2D map[58, 63].

One important feature in FTIRI study of cartilage is the recognition that the transition moments of amide I and amide Ⅱ are qualitatively perpendicular to each other in the context of the long axis of collagen in cartilage[59, 64-67]. Consequently, one can study the anisotropy of these spectroscopic absorptions in cartilage when the infrared light is polarized (Fig. 2f)), employing a similar concept as in $T_2$ anisotropy of μMRI[68] and retardation/angle calculations of PLM[44]. By using one or two polarizers in the optical path of infrared light in FTIRI experiments, all major absorption peaks in cartilage have been shown to possess depth-dependent anisotropies[60-62, 69]. In addition, through the establishment of an infrared spectroscopic library that characterizes the pure chemicals in the specimens, the amide absorption maps can be converted into chemical concentration maps in the tissue, via a factor analysis that utilizes all spectral data in multivariate analysis (e. g., principal component regression)[55, 58, 70-73]. The ability of measuring the changes in molecular concentration and fibril structures may prove important in identifying early signs of tissue degradation, such as collagen disruption and PG loss associated with pathological conditions in osteoarthritis.

### 1.4 Other imaging and non-imaging tools

In addition to the imaging techniques mentioned in the previous sections of this report, we have used several additional imaging and non-imaging techniques-each provides complementary and unique information of the structure and properties of cartilage during its early degradation.

Microscopic computer tomography (μCT) shares the same physics principles and engineering architectures as X-ray computer tomography (CT), a technique widely used in the hospitals. The image contrast in CT is mainly based on the attenuation of X-rays. In cartilage and bone specimens, bone, with its high mineral content, attenuates X-rays sufficiently to yield high quality images of the microstructures of the bone[74-75]. In comparison, articular cartilage (and other non-mineralized soft tissues) absorbs little X-ray. One approach to increase the contrast of cartilage in μCT is the use of contrast agents[76-84]. Since most contrast agents have specific charges, the equilibrium distribution of the ionic contrast agent in cartilage can be used to map the negatively charged proteoglycans in cartilage[76, 81]. μCT can also be combined with μMRI to study the bone lesions. For example, the high intensity regions in bone marrow in some $T_2$-weighted MRI images have been used to indicate bone marrow lesions, which represent bone marrow necrosis and fibrosis, trabecular abnormalities, and bone edema[85-86]. μCT is quickly becoming a useful tool in animal model research of cartilage and bone disease.

Electron microscopy (e. g., transmission electron microscopy, TEM) is the only imaging tool that has sufficient resolution to directly visualize ultrastructural objects such as individual collagen fibrils in cartilage[5, 87-93]. However, the significance of the fine structure of cartilage at the nano-scale can often be better understood as a part of its gross morphological architecture. This desire for both morphological and nano-scale knowledge motivated a 2D image analysis

procedure in our lab, a procedure which can quantify and summarize the graphical features of TEM images without losing the significance of the fine structures[94]. The procedure involves a localized 'vector' analysis that analyzes the power spectrum in reciprocal (Fourier) space. Three quantitative attributes essential to the biology and biomechanics of the tissue can be extracted, permitting direct correlations and comparisons among interdisciplinary techniques including μMRI, PLM, TEM and FTIRI[94].

The relationship between the depth-dependent structure and the mechanical properties of articular cartilage has long been of interest, since the ultimate measure of cartilage is its load-bearing ability. Investigations have involved the use of techniques requiring tissue to be substantially modified (i.e., enzymatically) from its fresh, functional condition. By using a technique that combines microscopic optical imaging and mechanical loading[95], we showed the possibility of visually tracking intra-tissue displacement of the chondrocytes to obtain quantitative depth-dependent stiffness measurements in healthy and degraded cartilage. Compressive resistance in cartilage increased with depth from the articular surface, confirming the concentration gradient of the negatively charged proteoglycan molecules in articular cartilage[31]. The understanding of intra-tissue load distribution in approximate in vivo conditions is of great importance in cartilage biology and medicine, potentially providing an important method for analyzing the coordinated changes in cartilage composition and function due to aging and disease.

## 2 Final remarks

The importance of imaging-derived data in OA research cannot be overemphasized[96-97]. In our laboratory, μMRI plays a central role since it is uniquely positioned to offer directly translational data to clinical MRI. In addition, we employ in our work PLM (which images the collagen organization based on the optical birefringence), FTIRI (which images the chemical concentrations based on the dipolar vibrations), and μCT (which images the interface between cartilage and bone). Even though neither μMRI nor PLM nor FTIRI nor μCT has the resolution to identify individual collagen fibrils or other molecules, a multidisciplinary approach in microscopic imaging can identify subtle changes in the morphological structure and molecular concentration of cartilage due to natural lesions and mechanical loading. This enables the monitoring and prediction of early changes in tissue that lead to cartilage degradation as a clinical disease. We show that most of the early events in joint degradation after the impact can be monitored by microscopic imaging studies of animal models.

In addition to the clinical relevance of our cartilage research, articular cartilage (together with bone and other tissues in the joint) is an ideal integrated system in interdisciplinary research. This is because the essential performance of the tissue and the organ (joint) is assessed at the biomechanical, functional, morphological, and clinical levels while the control of the tissue's performance is rooted deeply at the ultrastructural, molecular, cellular, and biochemical levels. The areas of potential focus of cartilage research are therefore numerous, ranging from tissue engineering and nanotechnology for replacement tissues, physical sciences for molecular and cellular interactions in healthy and lesioned tissues, imaging and image analysis in multiple digital platforms, and clinical diagnostics for the management of this disease, the number one cause of human disability. The multidisciplinary nature of our biomedical research has enabled the training of graduate students who gain critical abilities and diverse skills through carrying out applied scientific research in their own careers.

In summary, multidisciplinary imaging at microscopic resolutions offers a unique approach to study the complex events during tissue degradation in animal models; each technique can be purposely tailored to dissect one aspect of the complex mechanisms of tissue degradation with microscopic resolutions. The necessity of this multidisciplinary approach recognizes the complex nature of the biomedical problems in our research.

Because cartilage at different depths can have a unique combination of structures and properties (e. g., bio-electrochemical composition, solid/fluid interaction, morphological architecture, mechanical property), many aspects of these depth-dependent tissue properties can be interpreted beyond their usual meanings as measured. With this approach, we aim to provide a unique and clinical-relevant roadmap that has direct translational potential to clinical disease management. It is our ultimate goal that this bench-top research will lead to the successful detection and intervention of early joint degeneration in humans.

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