J Musculoskel Neuron Interact 2004; 4(4):365-368

Review Article

Hylonome

Molecular (and functional) imaging of articular cartilage

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Keywords: Cartilage, Imaging, MRI, Glycosaminoglycan, Arthritis

Considerable progress has been made over the past decade in developing imaging methods for quantifying cartilage morphology. Over the coming decades, these methods will likely be supplemented by non-destructive "molecular" or "biochemical" imaging by MRI to provide an adjunct or surrogate for the destructive histological and biochemical assays used today. In this talk, we will provide a brief overview of emerging strategies for assessing cartilage molecular state – i.e., its biochemical composition and architecture.

Importantly, the macromolecular status of cartilage is inextricably linked to the functional integrity of the tissue. In particular, studies suggest that solid/fluid volume fraction, collagen composition, molecular structure and organization, and GAG composition are key macromolecular features that they have interdependent effects on functional integrity of cartilage. Accordingly, these macromolecular features have been the focus of efforts to develop non-destructive imaging methods. Techniques under development range from those specific to a given biochemical species (water, collagen ultrastructure, and GAG) to those that appear through correlative studies to have image contrast that is modulated by a particular combination of macromolecules. Our talk will briefly comment on many of the methods that have been used, and then look in some depth at several.

Water content can be measured directly and specifically using proton density imaging. The practical challenge lies in the sensitivity, because the variation of water content across disease states is less than 5%. As noted below, there are some MR contrast mechanisms that can overemphasize this significant but small change in proton density – albeit at the cost of specificity.

Collagen orientation can be elucidated using microscopic MRI (μMRI) by measuring the angular dependence of T2 images. This angle-dependency is referred to as the magic angle effect, and it arises because the preferred orientation of the matrix (primarily collagen) confers a preferred orientation to the water dipoles that are probed by T2 imaging. Although there are many factors that can affect T2, the angle dependence of T2 (magic angle imaging) provides specific information about the macromolecular (collagen) ultrastructure.

Methods developed to measure GAG, sodium imaging and the dGEMRIC method take advantage of the fact the GAG is charged - in fact, they can more accurately be said to be assessing fixed charge (the charge "fixed" to the macromolecule, which in cartilage is essentially all due to GAG). These methods have considerable dynamic range and ensuing sensitivity to changes in [GAG] that one might observe *in vivo*. ([GAG] can range from around 60 mg/ml tissue water in normal native cartilage to less than 10 mg/ml in diseased, repaired, or engineered cartilage.) In addition, the well-developed biophysical understanding underlying the methods permits, in principle, a measurement that can be quantitatively related to [GAG]. Thus, charge-based methods for evaluating cartilage GAG are (or can in principle be) sensitive, specific, and quantitative.

Thus, in overview, proton density, magic angle, and charge-based imaging are each methods that are grounded in and supported by biophysical models that enable a predictable relationship between MR image and water content, collagen ultrastructure, and glycosaminoglycan concentration, respectively. This biophysical basis confers a relatively high specificity.

For obvious reasons, specific methods are appealing; however, in the long run it is likely that considerable insights and important clinical and pathophysiological information will be gained from methods that are straightforward and practical, and not necessarily entirely specific. It is also likely that, when combined with information from a high-specificity method, unambiguous conclusions about the biochemical state can be derived from otherwise non-specific methods. We therefore offer a brief mention of many of these approaches.

Among the earliest methods to be considered to provide

M.L. Gray has no conflict of interest. D. Burstein is a consultant for Pfizer, Inc.

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Accepted 4 August 2004

information about the biochemical status of cartilage are T2-weighted imaging strategies. Already noted is the fact that T2 anisotropy appears to also be strongly linked (mainly through the magic angle effect) to the apparent directional orientation of the collagen network that normally varies with articular cartilage depth. This effect gives T2-weighted images a multilaminar and striated appearance, similar to that observed by histologic and EM methods. In contrast to magic angle imaging (in which T2 is measured at multiple angles) which takes advantage of this effect to specifically resolve collagen ultrastructure, a T2 image taken at a single arbitrary angle will have an indeterminate dependence on collagen structure. Furthermore, T2 *per se* is also dependent on tissue composition.

A number of studies correlated T2 (and other MR parameters) to biochemical assays of cartilage *ex vivo*, generally finding the strongest correlations with tissue hydration or collagen content. In an effort to better understand the relation between T2 and composition, a few studies have examined T2 in model systems where composition could be varied systematically, revealing sensitivity to each of collagen, GAG, and solid volume fraction. The particularly strong sensitivity of T2 to tissue hydration has been successfully exploited to create a hydration image in regions like the patella, where the imaging protocol can minimize orientation effects.

The complexities of the T2 dependence on cartilage composition and architecture and on imaging parameters pose a real challenge in defining a normal appearance by MRI. And, because of its lack of specificity, it is not presently possible to attribute T2 differences to a specific biochemical feature. At the same time, it is quite clear that T2-weighted images are strongly influenced by the biochemical status of cartilage, so with further study and possible combination with other, more specific methods, important and robust uses of T2 for biochemical imaging may emerge.

Magnetization transfer and T1rho have been suggested as alternative approaches for revealing the macromolecular state of cartilage. The magnetization transfer (MT) effect appears to be dominated by the collagen component, and is affected by collagen concentration and structure/damage. T1rho has been shown to be dependent on GAG concentration with interventions designed to specifically alter GAG, but also has a dependence on collagen that is similar to that seen with MT. But further work remains to determine under what conditions these approaches might be most useful.

To summarize, there are a wide variety of approaches that provide image contrast influenced by cartilage macromolecular composition. For most of these the specificity of changes in image contrast in reflecting a specific biochemical state remains to be elucidated. However, a few appear to provide very specific information. While none of these techniques are presently in routine clinical use, emerging data provide promise that the future will see patient-specific biochemical analysis of cartilage – an outcome almost unimaginable 20 years ago.

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