Spectroscopic Imaging of Cryopreservative Concentration and Diffusion in Cartilage

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Introduction: Cryopreservation is the process whereby cells or whole tissues are preserved by cooling to low sub-zero temperatures, typically -80°C or colder. At these low temperatures the biochemical processes that would lead to cell death are effectively stopped, allowing storage and future use or transplantation. While low temperatures promote preservation, the processes of freezing can kill cells. Cryoprotective compounds are commonly used to reduce damage from the formation of ice. A significant challenge facing low temperature tissue preservation is the design of schedules for cryopreservative loading concurrent with temperature changes that will ensure cell survival. Previously, conventional imaging techniques have been used to study cryopreservative diffusion in tissue samples[1,2], but without spectroscopic resolution of water and the cryopreservatives. In this study, spectroscopic magnetic resonance imaging is investigated as a direct measure of concentration and diffusion of both water and cryopreservative in articular cartilage, an aggressively targeted tissue for preservation.

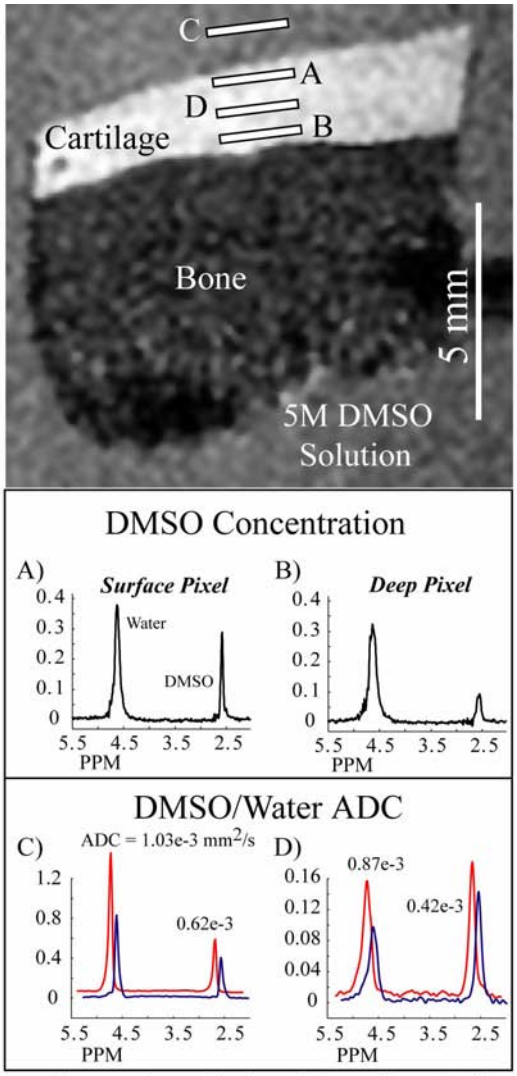
Methods: Samples of pig articular cartilage were loaded with the common cryopreservative solution dimethyl sulfoxide (DMSO) at 15°C. The cartilage samples (1 cm plugs) were harvested and placed in a 5M DMSO solution 45 minutes prior to imaging. All experiments were performed at 1.5 T (Siemens Sonata) using an echo-planar spectroscopic imaging pulse sequence with diffusion weighting, designed in house. A spin-echo line-scan variation of the pulse sequence was used to allow spectra to be acquired from a line, in a single shot. The line was oriented perpendicular to the bone-cartilage interface. Parameters: 300 Hz spectroscopic bandwidth, 2 mm slice thickness and .3 mm spatial resolution along the line, TR = 6 seconds, b = 565 s/mm^2. TE = 60 ms for diffusion experiments and TE is varied from 8 ms to 200 ms in 16 steps for T_2 studies, used to correct T_2 signal loss for the calculation of concentration. Total acquisition time was ~2 minutes. A 3 cm diameter surface coil was used for signal reception.

The goal of these preliminary experiments was to determine the feasibility of measuring cryopreservative concentration and the apparent diffusion coefficients in articular cartilage from spectroscopic images. An automated spectral fitting routine was used to quantify the chemical shift, line width and yield of the water and DMSO peaks.

Results: The figure shows a gradient echo image of a pig articular cartilage sample. The signal void on the right is the sample holder, which is inserted into the bone to suspend the sample in the DMSO solution. Spectra are shown from pixels at the surface and near the bone-cartilage interface (TE = 8 ms) acquired 45 minutes after the sample was immersed in the 5M DMSO solution (15°C). Quantitative spectral fitting yielded concentrations of 4.88M at the surface (A) and 1.80M in the deep pixel (B), illustrating a significant concentration gradient at this time-point in DMSO loading. Sample spectra from diffusion weighting experiments indicate a significant difference between water and DMSO ADC values both in solution (C) and in a sample tissue pixel (D). Water signal intensity is significantly reduced in cartilage compared to DMSO due to shorter T_2 values.

Conclusion: Preliminary studies show that echo-planar spectroscopic imaging can provide sufficient spatial resolution and signal to noise for quantification of cryopreservative concentrations as well as water and cryopreservative ADCs in target tissue samples. Also, all proposed measurements are feasible on a standard clinical 1.5T system. Quantification of ADC, T_2 and relative spin density (concentration) can be achieved in as fast as 2 minutes for a line-scan acquisition. Two-dimensional spectroscopic imaging can also be implemented, although with longer scan times. Spectroscopic imaging as opposed to conventional imaging allows direct quantification all species present, and will allow multi-component solutions or scalar-coupled spin systems, such as sugars, to studied.

In the short term, studies will be repeated throughout complete cryopreservative loading experiments (several hours) to determine the temporal and spatial variations in concentration and ADCs. Measured diffusion values and concentrations will also be used in tissue diffusion models, which allow complex loading protocols to be simulated given the solution concentrations and the measured ADC values. Because practical loading studies take place over a range of decreasing temperatures, the influence of temperature on water and cryopreservative diffusion will be included in future experiments.



A gradient echo image of a pig articular cartilage plug (in a 5M DMSO solution) shows sample pixels locations from a spectroscopic imaging experiment. Surface and deep pixels locations in A) and B) show a large DMSO concentration gradient (45 minutes of soaking). Diffusion studies compare the raw spectra used for ADC calculation, both in the solution (C) and in cartilage (D).

References: [1] Isbell SA, et al. Measurement of cryoprotective solvent penetration into intact organ tissues using high-field NMR microimaging. *Cryobiology* **35**, 165-172 (1997). [2] Carsi B, et al. Cryoprotectant permeation through human articular cartilage. *Osteoarthritis and Cartilage* **12**, 787-792 (2004).