Investigation of the influence of the extracellular matrix on water diffusion in brain and cartilage

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Synopsis

Water diffusivity in biological tissues can be related to the underlying microstructure that modulates the restricted or hindered diffusion, and can be studied with NMR experiments. The extracellular matrix, whose composition depends on the tissue type, may have an influence on diffusion. In this work we study the influence of the extracellular matrix on diffusion, by measuring brain and cartilage samples before and after the enzymatic removal of the extracellular matrix components. The activation energy for the self-diffusion of water seems to be not significantly affected by the treatment for brain tissues.

Purpose

Diffusion of water in biological tissues is sensitive to the underlying microscopic structure because macromolecules act as obstacles or sources of electrostatic fields that hinder or restrict the diffusion paths. The diffusion behavior in tissues is known to be non-Gaussian. Moreover, two different water regimes (with distinct activation energies) have been found in brain tissue and assigned to a relatively 'free', bulk-like compartment and water in contact with macromolecules [1,2].

Due to its macromolecular composition, we may thus assume that the extracellular matrix (ECM) component of tissues impacts water diffusion. For cartilage, the ECM is already known to play a role in the generation of MRI contrast [3] and to affect diffusion [4]. Despite its very different structure [5-7], it has been recently suggested that the ECM may also influence the MR signal in brain [8], and iontophoretic studies indicate an influence on ion diffusion in rat brain [9].

To more directly assess an ECM influence on water diffusion, we performed a series of experiments in post-mortem brain tissue samples before and after enzymatic removal of the ECM. Additional experiments were performed in cartilage, whose hyaluronan content is remarkably higher compared to the brain.

Methods

Two paraformaldehyde-fixed specimens from goat thalamus and another two from dog ear were collected in 7mm7mm-diameter glass tubes and stored in 0.1M, pH 7.4 Phosphate-Buffered Saline (PBS) solution at 4°C. Each one of the brain and cartilage samples, were treated with hyaluronidase from bovine testis (Sigma H3884), with up to ≈4500units/ml, at 37°C for 14days, to completely remove the hyaluronan-based ECM. The hyaluronidase-digested cartilage specimen was subsequently (after diffusion MR) also treated with 2% collagenase (Fluka) at 37°C for 14days to separately digest collagen ECM components. The remaining two untreated samples were used as reference to asses potential changes during storage.

Non-localized PFG diffusion measurements were performed on a custom-built FEGRIS NT 125MHz125MHz spectrometer [10] with a gradient amplitude up to 35000mT/m along the main magnetic field. It permits to achieve very short diffusion times ($\Delta = 2$ms; $\delta = 0.6$; $T_E = 4$ms; $TR = 3$s) to minimize restriction and exchange effects. The b-values were varied in 20 steps (up to 18000s/mm^2), and the temperature was varied between 20°C and 0°C in steps of 2°C. Additionally, T_1 was measured using an inversion-recovery sequence. Diffusion data were fitted to a bi-exponential model, while a mono-exponential fit was used for obtaining T_1. Activation energies and the pool size fractions were extracted from Arrhenius plots.

After the MR acquisitions, the brain samples were sliced and stained for hyaluronan-sensitive markers.

Results

Arrhenius plots for both the brain and the cartilage samples are shown in Fig. 1. Activation energies and pool size fractions, as well as T_1 results are summarized in Fig. 2. It was not possible to determine the activation energy for a 'slow' water compartment in cartilage due to the relatively low signal contribution. T_1 was decreased by ≈10% to ≈20% after hyaluronan digestion both in thalamus and cartilage.

Diffusion coefficients and activation energies of both water fractions did not show a measurable change upon treatment in the thalamus sample. Staining results (Fig. 3) confirm that the hyaluronan digestion was effective in this sample. In cartilage, the diffusion coefficient decreased by ≈7% after hyaluronan digestion but increased by ≈15% following collagenase treatment, while the activation energy was only marginally affected.

Discussion

The observed T_1 decrease upon treatment in the thalamus sample is consistent with previous imaging results [9]. However, the underlying microstructural variation is not reflected in a measurable effect on water diffusion. The cartilage sample did indicate an influence on water diffusion from the ECM, which might be explained by the substantially higher hyaluronan content in this tissue type.

Interestingly, the diffusivity decreased after hyaluronidase treatment, which might indicate that macromolecular fragments remain after digestion as obstacles to slow down water mobility. Such fragments might be removed upon subsequent collagenase treatment as

indicated by an increase in water diffusivity. Due to the lower ECM content in brain tissues, the effects on diffusion might be too small to be detectable unless high spatial resolution is achieved to better study the quite heterogeneous distribution between different brain regions.

Conclusion

We did not observe a significant effect on water diffusion in fixed brain tissue upon removal of the hyaluronan-based ECM in experiments employing ultra-high magnetic field gradients.

Acknowledgements

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Figures

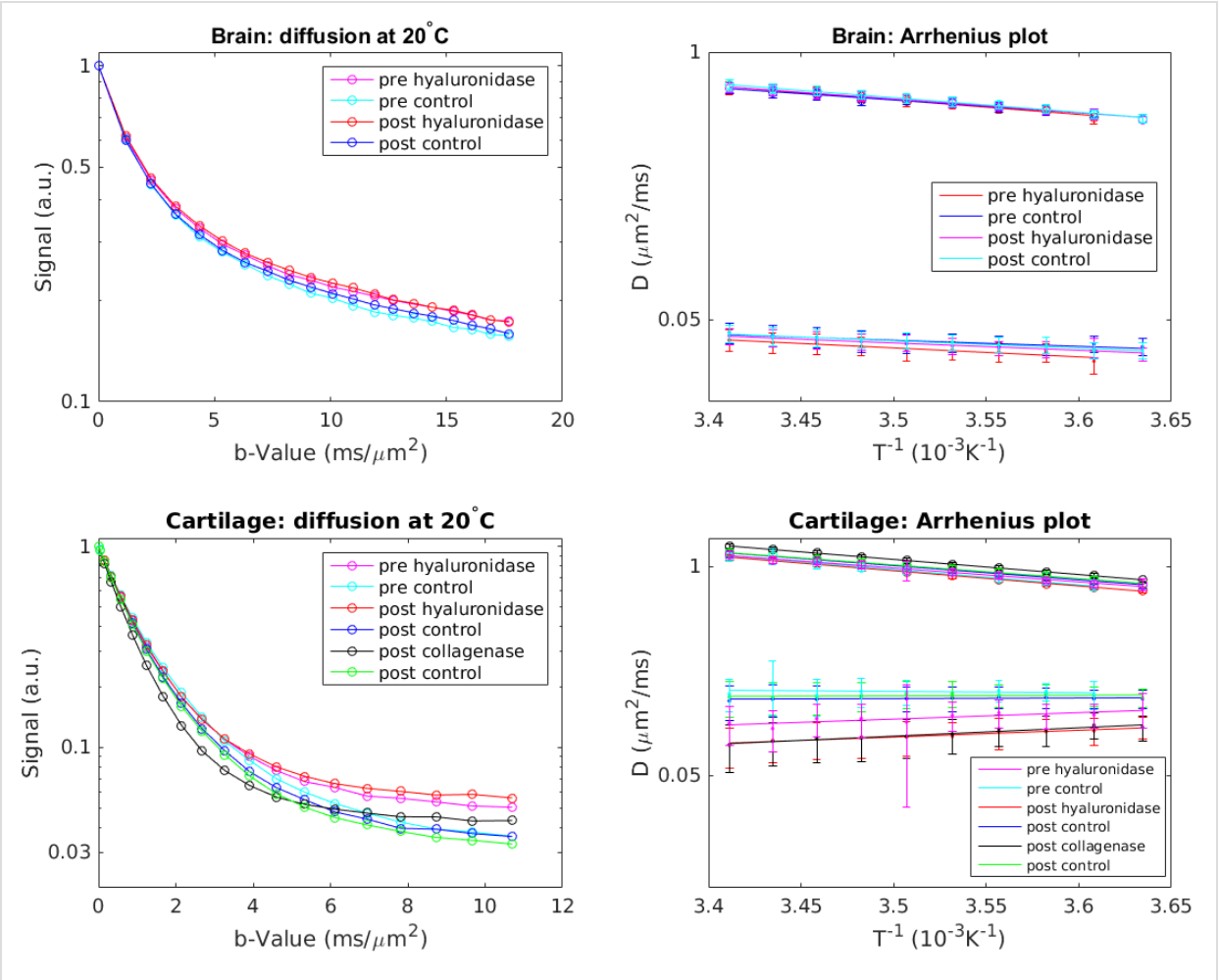

Fig. 1: Diffusion decays at 20°C and Arrhenius plots .

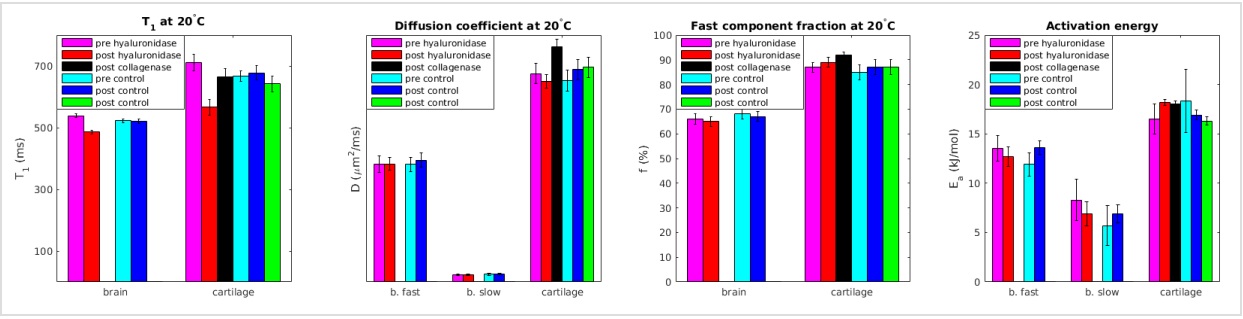

Fig. 2: Histograms of results from *T1* and diffusion measurements for the different samples and experimental conditions.

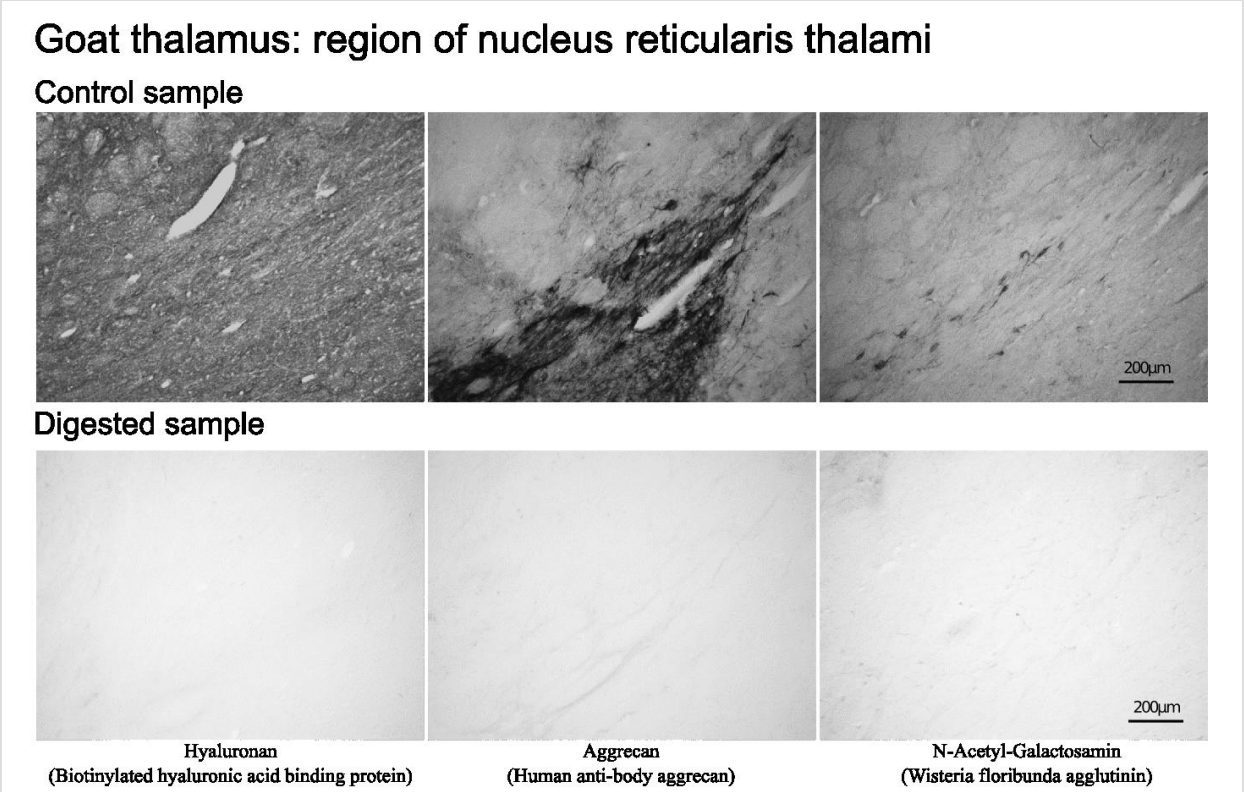

Fig. 3: Evaluation of the efficacy of hyaluronan digestion in the thalamus sample (upper row: undigested reference sample; bottom row: digested sample).