Possible Contribution of the Extracellular Matrix to the MRI Contrast in the Brain

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Target audience: Researchers and clinicians interested in quantitative MRI and brain tissue microstructure.

Purpose: The tissue composition of the brain can be related to different image contrast sources in quantitative MRI. Particularly, myelin and iron are considered to be major sources of MRI contrast and recent studies have shown a direct relationship to T_1 and T_2*, respectively [1]. However, other tissue components may play a role in the MRI contrast, including the ExtraCellular Matrix (ECM) which constitutes up to 20% of total brain tissues. Previously, it was shown that ECM contributes to contrast both in synthetic samples [2] and connective tissues [3,4]. The ECM of brain tissues is composed primarily of hyaluronan polymer chains, which are anchored via hyaluronan synthase in the membrane of cell somata and proximal dendrites. The specialized neuronal ECM of Perineuronal Nets (PNs) additionally provides an accumulation of Chondroitin Sulfate ProteoGlycans (CSPGs), which bind to the hyaluronan chains. Link proteins stabilize the interaction between hyaluronan and CSPGs. Finally, tenascin-R binds to CSPGs forming a quaternary complex surrounding neurons. The CSPGs are characterized by GlycosAminoGlycan side chains (GAG), which have a strong negative charge and are hypothesized to determine the physiological function of the neuronal ECM/PNs [5,6]. In order to investigate the ECM contribution to MRI contrast in brain tissues, we performed pilot experiments on a human post-mortem sample with heterogeneous ECM content, including scans before and after the digestion of the ECM component.

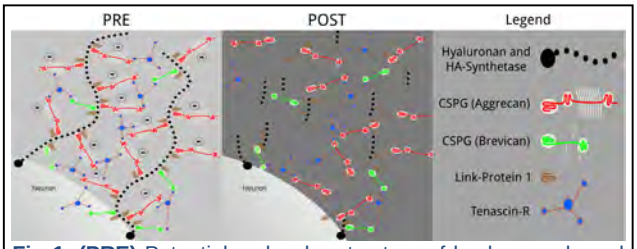


Fig 1. (PRE) Potential molecular structure of hyaluronan-based perineuronal and perisynaptic ECM components and **(POST)** the assumed effect of hyaluronidase digestion.

Methods: The hyaluronan-based ECM of the CNS, and also the specialized neuronal ECM of PNs, can be effectively removed by digesting the tissue with hyaluronidase and, thereby, disassembling the macromolecular ECM scaffold, as shown in Fig. 1 [7]. A post-mortem sample, fixed with 4% ParaFormAldehyde (PFA), of ~4×2×1 cm from left thalamus and midbrain (Fig. 2) of a deceased male donor (age: 82y, COD: colon carcinoma, with no neuropathological abnormalities) was measured before and after ECM digestion. The digestion was performed with hyaluronidase (from bovine testis, Sigma H3884, with up to ~4500 units/ml, in PBS pH7.4 at 37 °C for ~14 days). For each MRI scanning session, the sample was prepared by flushing it in PBS 0.1M with pH of 7.4 for ~2 days, and then submerging it in perfluoropolyether, after placement in a spherical container. The sample was immobilized by two sponges, to which a layer of paraffin was applied. Images of the sample were acquired using a 3T MedSpec 30/100 scanner (Bruker, Ettlingen, Germany) and a custom-built Helmholtz coil with highly homogenous B_1 field [8]. During each session, quantitative T_1 and T_2* maps (0.3mm isotropic nominal resolution) were recorded. The T_1 maps were obtained from multiple 3D FLASH measurements (TE: 6 ms) with variable flip angle α (10, 20, 30 deg) and repetition time TR (30, 90, 270 ms), which were then used to perform a non-linear fit to the signal. The T_2* maps were obtained from multi-echo FLASH measurements (α: 40°; TR: 270 ms; TE: 6, 15, 24, 33 ms) using a log-linear fit. Each set of experiments lasted ~7 days, and the time between pre- and post- sessions was ~4 weeks. Contributions to MRI contrasts were assessed by visual inspection of the maps and the voxel-by-voxel correlation histograms.

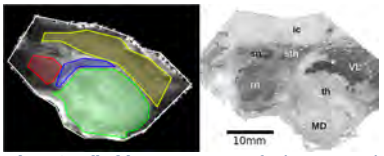
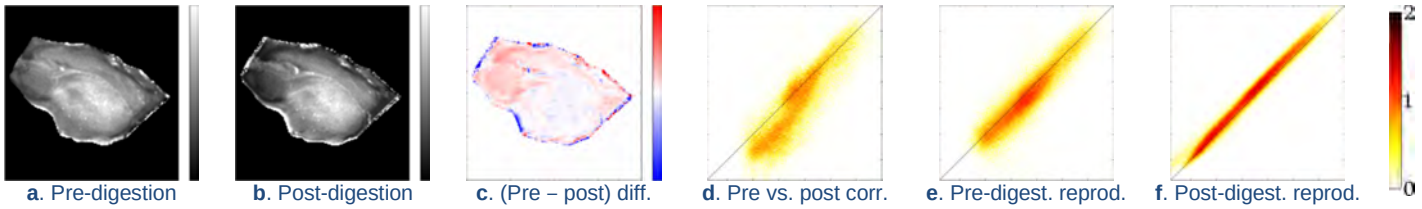


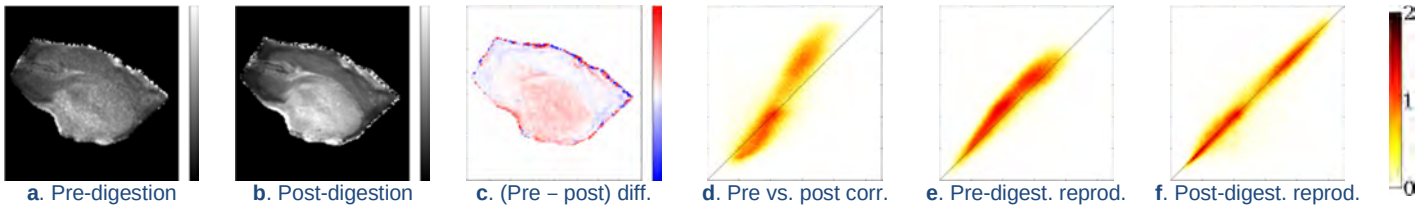
Fig. 2. (left) Anatomy of the sample: green is thalamus (th); blue is subthalamic nucleus (sth); red is red nucleus (rn); yellow is internal capsule (ic). Sth and rn have high ECM content. **(right)** ECM-stained histology showing the same tissues as the sample.

Results: T_1 and T_2* maps for pre- and post-digestion experiments are shown in Fig. 3a, 3b, 4a, 4b, alongside with their difference (Figs. 3c, 4c), voxel correlation histograms (Figs. 3d, 4d) and test-retest reproducibility histograms of results obtained in subsequent scans under identical digestion conditions (Figs. 3e, 3f, 4e, 4f). Visual inspection of both T_1 and T_2* maps show changes in contrast across regions. This observation is further supported by the difference and correlation analyses, and is consistent through the 3D volume (i.e. not limited to the shown slice). The deviations from the identity line in different value ranges are significant, when compared to test-retest results, indicating that this behavior is specific to the digestion procedure.



a. Pre-digestion **b**. Post-digestion **c**. (Pre – post) diff. **d**. Pre vs. post corr. **e**. Pre-digest. reprod. **f**. Post-digest. reprod.

Fig. 3. T_1 mapping (gray-scale values from 60 to 400 ms; blue-to-red-scale values from -340 to +340 ms; 2D histograms with 500 bins in log10 scale).



a. Pre-digestion **b**. Post-digestion **c**. (Pre – post) diff. **d**. Pre vs. post corr. **e**. Pre-digest. reprod. **f**. Post-digest. reprod.

Fig. 4. T_2* mapping (gray-scale values from 10 to 65 ms; blue-to-red-scale values from –55 to +55 ms; 2D histograms with 500 bins in log10 scale).

Discussion: Significant contrast changes are observed for both T_1 and T_2* upon removal of the ECM. However, the observed patterns across regions are complex. For example, T_2* values in the red nucleus (rn) and subthalamic nucleus (sth) are approximately constant but decrease in thalamus (th) after digestion, while T_1 values become longer in rn and sth, but not markedly affected in th. The T_1 changes seem to agree with previous observations in synthetic samples [2]. However, the pre-digestion T_1 map does not simply reflect the expected ECM content due to additional contrast contributions, e.g., from myelin. Moreover, we cannot exclude that other mechanisms, such as partial tissue decomposition during digestion and slightly inconsistent temperatures, might have had an impact on the observed changes. Although further experiments are required to address such current limitations, the results from our pilot study seem to support the hypothesis that ECM component(s) contributes to MRI contrast based to relaxation parameters.

Conclusion: While further experiments are required to investigate the role of ECM in the generation of MRI contrast and underlying biophysical mechanisms, the observed contrast changes after ECM digestion seem to indicate that ECM affects relaxation times in brain tissue.

References: [1] Stüber et al. NeuroImage 93: 95-106 (2014); [2] Laurens et al. Skeletal Radiol. 41(2): 209-217 (2012); [3] Nishioka et al. JMRI 35(1): 147-155 (2012) [4] X. Li and S. Majumdar, JMRI 38(5): 991-1008 (2013); [5] Morawski, et al. Philos Trans R Soc Lond B Biol Sci. 369:1654 (2014); [6] Morawski, et al. Int J Biochem Cell Biol. 44(5): 690-3 (2012); [7] Happel, et al., PNAS 111(7): 2800-2805 (2014) [8] R. Müller et al. Proc. ISMRM 21: 4239 (2013).

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