Ferritin Mineral Core Composition in Health and Disease

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Iron (Fe) is an essential metal involved in a wide spectrum of physiological functions, including oxygen transport and enzymatic reactions. Maintenance of iron homeostasis is regulated in part by iron confinement, mineral composition and its oxidation state. Ferritin is the major iron storage protein found in mammalian tissues comprising of a nanoscale iron core with up to 4500 iron atoms in the form of ferrihydrite. Fe oxidation state and composition of the ferritin core can govern its ferroxidase activity and thus the storage and transport of iron in health and disease.

Thus far limited studies elucidate the nature of the Fe core of pathological ferritin. Significant differences between the mineral composition of physiological vs. pathological ferritins have been found in chronic neurodegenerative diseases by analyzing the crystallinity and oxidation state of iron cores by analytical transmission electron microscopy (TEM)[1], [2]. While physiological ferritin is understood to consist primarily of Fe(III) rich ferrihydrite, pathological ferritin was reported to be abundant in Fe(II) containing magnetite [3], [4] (a mineral with significantly higher magnetism than ferrihydrite).

In this study we aim to identify the mineral composition of pathological vs. physiological ferritin in instances of acute up-regulation of iron and ferritin. We hypothesize that pathological ferritin will differ from physiological ferritin in mineral content, composition and in tissue distribution, which in turn will impact the magnetic properties of its iron core. A well-characterized rodent model of spinal cord injury exhibiting iron and ferritin up-regulation was used along with analytical transmission electron microscopy (TEM) and magnetic force microscopy (MFM) to examine the composition of ferritin cores.

All animal surgeries were performed in agreement with The Ohio State University Institutional Animal Care and Use Committee. Adult Sprague Dawley rats were injured at the T8 vertebral level with a moderate (200 kDyne force) spinal contusion using the Infinite Horizons device (Precision Instruments). For tissue collection, rats were deeply anesthetized with a ketamine/xylazine mixture and then perfused transcardially with distilled water followed by 250 ml of 4% paraformaldehyde 2% glutaraldehyde in 0.1 M cacodylate buffer. Spinal cords were removed, immersion-fixed overnight at 4°C, and placed in 0.2 M cacodylate buffer for storage. Tissue was processed for TEM analysis, beginning with a brief post-stain in 1% osmium tetroxide. All other heavy metals and stains were avoided in the sample preparation to avoid loss of signal collection efficiency for analytical TEM.

Iron and ferritin upregulation was found within the lesions of injured spinal cords by Perls staining and immunohistochemistry. An FEI Titan S/TEM operating at 300 keV was used to detect Fe in the spinal cords of injured rats. Energy-Dispersive X-ray Spectroscopy (EDS) and Electron Energy Loss Spectroscopy (EELS) analysis confirmed the presence of Fe (figure 1). Further, EELS analysis indicated

the presence of Fe(II) within the spinal cord lesion. Naïve rat spinal cords were evaluated by conventional TEM along with a Perls Iron assay, no ferritin or iron could be detected in naïve spinal tissue respectively (data not shown).

For MFM analysis, spinal cord tissue was embedded in optimal cutting temperature (OCT) medium and cross-sections were cut at 10 µm on a cryostat and mounted onto poly-lysine coated glass. Adjacent sections were taken onto glass coverslips for Perls stain analysis.

MFM imaging was performed on injured spinal cord within regions of low and high Fe deposition. As identified by utilizing the adjacent Perls stained sections as maps to locate the regions of interest. At lift heights of 30 nm a strong positive phase shift is present, while sample topography is no longer visible at 30 nm.

Our studies demonstrate the use of analytical high-resolution microscopy approaches for iron detection in biological tissues for understanding its oxidation state and tissue distribution. These insights would help enhance our understanding of the role of iron in health and disease.

References:

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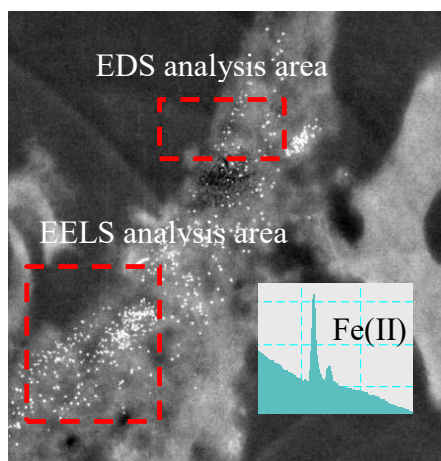
Figure 1. STEM image of rat spinal cord shows ferritin within cytoplasmic region of a cell. EDS and EELS indicate the presence of Fe.
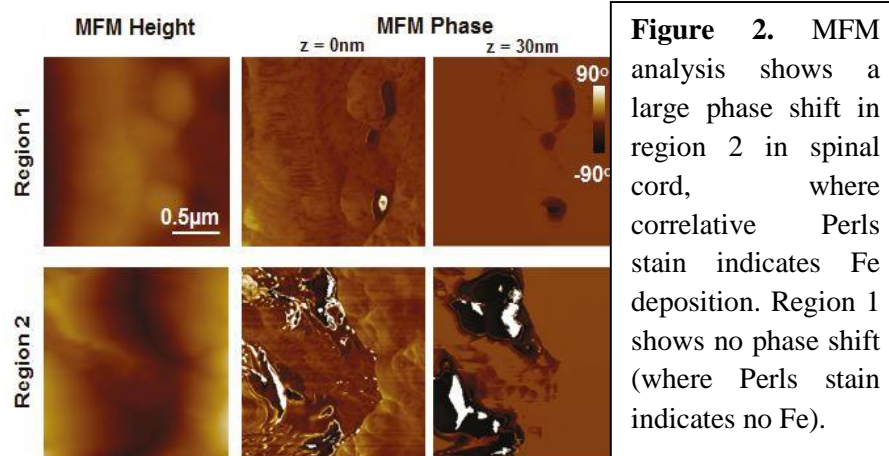
Figure 2. MFM analysis shows a large phase shift in region 2 in spinal cord, where correlative Perls stain indicates Fe deposition. Region 1 shows no phase shift (where Perls stain indicates no Fe).