

Supplementary Material

Correlative Microscopy to Localize and Characterize Iron Deposition in Alzheimer's Disease

Supplementary Methods

FIB liftout procedure

A protective layer of carbon, roughly 500 nm thick, was deposited over the top surface of the region to be extracted from the sample to reduce surface damage from the ion beam. This procedure was performed in the FIB-SEM using the ion beam and a carbon-containing precursor gas, naphthalene, and is commonly used in FIB preparation of TEM samples [1]. Trenches were milled on either side of the carbon coated region using a 2.5 nA 30 kV ion beam. The material left between the two milled regions was a lamella of approximately 1-2 μm thickness. Once this was completed, the sample was tilted back to 0° with the sample normal pointed toward the electron beam column.

A micromanipulator needle (Omniprobe, Oxford Instruments) inside the FIB-SEM was positioned $<1 \mu\text{m}$ from the top surface of the region of interest. The needle was connected to the surface of the sample by the deposition of platinum from methylcyclopentadienyl (trimethyl) platinum using the ion beam. Finally, three rectangular milling regions of interest were drawn in a U-shape to separate the block from the surrounding tissue. A reduced ionbeam current, 80 pA, was applied for this step.

The manipulator, with the lamella attached, was retracted, and the microscope sample chamber was vented. An Omniprobe TEM grid, consisting of a 3 mm diameter semicircle of copper with several thin posts protruding, was loaded onto the stage and the microscope was pumped down again. The manipulator was used to position the lamella close to the edge of one of the posts ($<1 \mu\text{m}$), and additional carbon was deposited to attach the two. The manipulator

was cut away with the ion beam (30 kV, 80 pA) to leave the sample as a cantilever mounted to the copper post.

At this point, the sample was still 1-2 μm thick, unsuitable for TEM. The ion beam was used again further thin the sample. The beam was run at 30 kV and 80 pA. The “cleaning cross section” function on the FIB-SEM was applied to remove material from the top and bottom surfaces of the lamella. A final clean-up mill was performed at 5 kV and 15 pA after the sample was estimated to reach electron transparency (approximately <200 nm was found to be acceptable for imaging at 300 kV due to the relatively low density of specimen). As the sample thins, care must be taken to minimize bending of the cantilever. In particular, imaging with higher electron beam currents (above about 0.1 nA at 2 kV) was found to cause the sample to bend under its own weight, likely due to heating.

STEM

The microscope was operated in STEM mode and run at 300 kV. Annular dark field (ADF) STEM imaging was performed using the DF detector on the Gatan Quantum GIF, using a camera length of 38 mm. Under these conditions, electrons scattered through from 40-70 mrad are collected by the detector to form an image.

STEM-EELS

The microscope was operated at 300 kV under the following conditions: monochromator off, spot size 7, 50 μm C2 aperture, 38 mm camera length, 2.5 mm GIF aperture. These conditions correspond to a convergence angle of 9.3 mrad and a collection angle of 18.7 mrad. The pixel size was 0.15 μm . A dispersion of 0.5 eV was selected to collect data over the range of 550 eV to

1550 eV, allowing the Fe L (708 eV) and Zn L (1020 eV) edges to be obtained simultaneously [2]. The pixel time was 0.5 s, and the entire scan took roughly 30 min to complete a 45x40 pixel spectrum image. STEM-EELS chemical maps were generated as follows. The spectra were background subtracted by power law fitting about 100 eV of the pre-edge background in Gatan DigitalMicrograph software (Gatan, Pleasanton, CA). The integrated intensity under the peak (over the ranges 716-726 eV for Fe L and 1085-1230 eV for Zn L) was calculated at each pixel to assign an intensity, a normal procedure. The integration window for zinc was wider than that for iron because the zinc edge demonstrates a delayed maximum without a sharp peak [2].

Monochromated STEM-EELS

The microscope was operated under the following conditions: C3 aperture 30 μm , camera length 48 mm, spot size 15 μm . These conditions result in a convergence angle of 5 mrad and a collection angle of 14.6 mrad. Dual-EELS was employed to collect core-loss (Fe-L edge) and low-loss, or zero loss, peak (ZLP) spectra together. Each of the core-loss spectra was collected with an integration time of 5 s, the latter with an integration of 0.0025 s. This allows each spectrum to be aligned relative to the ZLP to determine the exact energy loss more accurately.

REFERENCES

- [1] Giannuzzi LA, Stevie FA (1999) A review of focused ion beam milling techniques for TEM specimen preparation. *Micron* **30**, 197-204.
- [2] Ahn CC, Krivanek OL, Disko MM (1983) *EELS atlas: a reference collection of electron energy loss spectra covering all stable elements*. HREM Facility, Center for Solid State Science, Arizona State University.

Supplementary Table 1. Demographics of AD patients analyzed in this study.

Patient	Age at death	Gender	Braak and Braak Stage	Postmortem Interval (h)	Secondary Disease
A1	87	F	V-VI	32.48	Mild cerebrovascular disease
A3	84	F	V-VI	32.52	Cerebrovascular disease
A4	89	M	V-VI	37.14	Atherosclerosis, severe
A5	83	M	V-VI	15.00	No other pathology

Supplementary Table 2. Sequence parameters used for ex vivo MRI acquired in this study.

Sequence	3D bSSFP	GRE
Resolution (mm)	0.0586x0.0586x0.1	0.0586x0.0586x0.1
Matrix size	512x512	512x512
Slices	256	256
TR (ms)	21	21
TE (ms)	20	20
Flip Angle (degrees)	20	20
Bandwidth (kHz)	8	8
Phase cycles	8	N/A
Number of excitations	N/A	8
Acquisition Time	3 h 37 min	3 h 37 min