Visualization and Analysis of Superparamagnetic Ferrogels as Carriers for Therapeutical Molecules to the Inner Ear

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We have developed a new type of ferrogel consisting of superparamagnetic iron oxide nanoparticles (SPION) and Pluronic® F127 (PF127) copolymer. Pluronic copolymers possess a unique viscosity-adjustable property which makes PF127 gels easy to handle compared to conventional cross-linked hydrogels based on, amongst others, poly(vinyl alcohol), gelatin, poly(*N*-isopropylacrylamide). Hydrophobic drugs can be loaded in the ferrogel as a "sol" at low temperature and the drug loaded "sol" can be injected into tissues. After injection at the human body temperature, the "sol" rapidly becomes a gel that acts as a matrix for releasing therapeutic drugs. The release rate of the drug from the ferrogel can be tuned with an external magnetic field. These ferrogels were tested in a human temporal bone as well as in the organotypic culture of mouse inner ear. Iron oxide nanoparticles were visualized in histological sections by light microscopy using the histochemical Berlin-Blue reaction. It demonstrates Fe^{2+} and enables lower magnified overviews of tissue incubated with iron containing ferrogels. This technique can be applied on epoxy resin embedded as well as in paraffin embedded tissue.
Ultrathin sections of mouse cochlear organotypic culture and human temporal bone were visualized by means of energy filtered transmission electron microscopy (EFTEM). Iron oxide nanoparticles from the ferrogel appeared electron dense in zero loss imaging, but in a conventionally prepared and stained ultrathin section the particles could not be distinguished clearly from any other electron dense compartment stained with lead citrate. EFTEM Libra 120® allows easy imaging of unstained sections and imaging at 250 eV of energy loss (High Contrast Imaging, HCI) clearly emphasized the nanoparticles (Figure 1a). In addition energy filtered TEM allows elemental analysis with high spatial resolution. Parallel electron loss spectroscopy (PEELS) proofed the presence of iron oxide in the particles (Figure 1a insert, Figure 2b). Furthermore the spatial elemental distribution of iron could be visualized with electron spectroscopic imaging (ESI). Due to the compositional information provided by energy filtered TEM, the iron oxide nanoparticles in the ferrogel could be detected doubtlessly. Since the particles are very small, only energy filtered TEM in ESI mode can resolve the fine structural distribution of iron and directly correlate it with the ultrastructure of the tissue (Figure 1b, Figure 2a).

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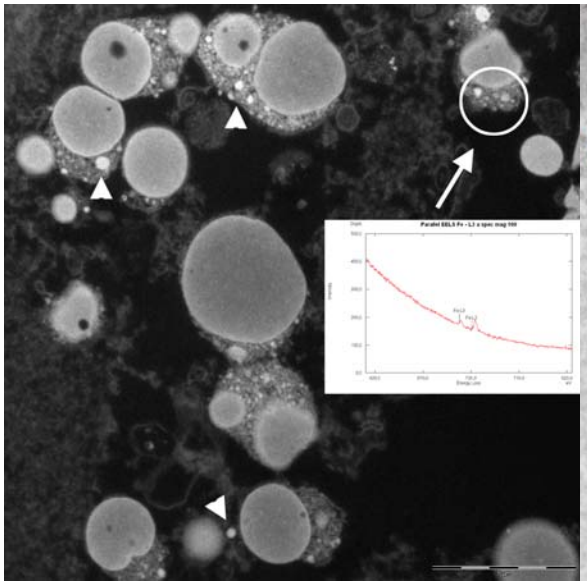

Figure 1a

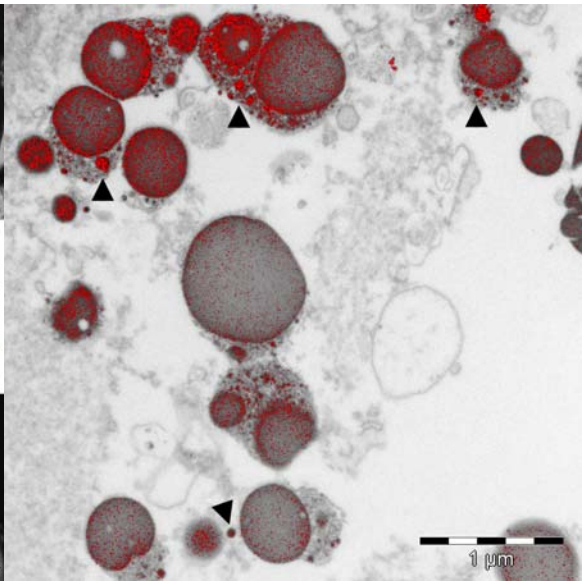

Figure 1b

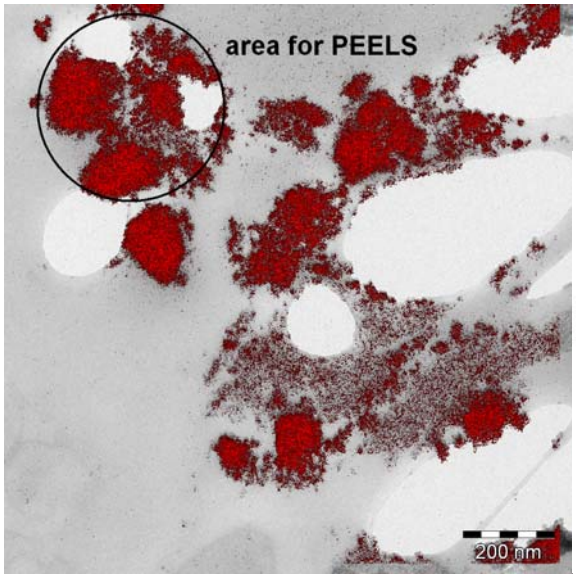

Figure 2a

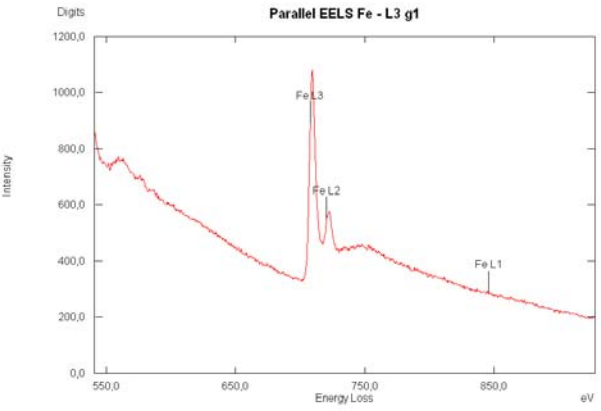

Figure 2b

Figure 1. EFTEM imaging of ultrathin sections of mouse cochlear organotypic cultures
a: EFTEM imaging at 250 eV of energy loss (HCI) clearly emphasizes the ferromagnetic nanoparticles (see arrowheads). Ultrathin section is completely unstained.
b: By means of ESI and EELS (Figure 1a insert), the particles could be identified to contain high amounts of iron (red). Ultrathin section is completely unstained.

Figure 2. EFTEM imaging of ultrathin sections of human temporal bone
a: Elemental distribution of nanoparticles containing iron (red) using ESI (3 window method). Ultrathin section is completely unstained.
b: PEELS of nanoparticles shows a clear signal of Iron and Oxygen (not shown). Ultrathin section is completely unstained.

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