From Synthetic to Natural Nanoparticles: Monitoring the Biodegradation of SPIO (P904) into Ferritin by Electron Microscopy

Juan D. López-Castro,1 Adrian V. Maraloiu,2,3 J. J. Delgado,1 J. J. Calvino,4 M.-G. Blanchin2 Natividad Gálvez4 and José M. Domínguez-Vera4,*

Electronic Supporting Information

Experimental Section

Ex vivo murine samples taken from ApoE-/- mice (C57BL/6 background) received from Guerbet Laboratories (Paris, France) were used in this study. ApoE -/- mice lack the apolipoprotein E, which has a key role in the normal catabolism of triglyceride-rich lipoprotein constituents. ApoE -/- mice develop normally but exhibit five times normal serum plasma cholesterol and spontaneous atherosclerotic lesions.

Animals were started on a high-fat diet (Western Diet, 0.5% cholic acid and 1.25% cholesterol) at 6 weeks of age. At 28 weeks of age, the animals were administered the P904 contrast agent at a dose of 1000 μmol Fe/kg body wt, a high dose to ensure success of trials in atheroma plaque imaging. Two remaining animals were not injected and considered as the control group.

While under deep anesthesia, 2 mice per time point (1, 15 and 30 days) were sacrificed by cervical dislocation and samples of the spleen were taken for Electron Microscopy analysis. Small sections of spleen samples from the sacrificed animals were cut and fixed in the fixer (1.5% glutaraldehyde and 1%paraformaldehyde in buffer 0.15M cacodylate). After fixation, the tissues were washed, dehydrated in alcohol and included in Epon resin which was finally polymerized. Ultrafine (a few ten nm thick) sections were obtained from the polymerized specimens by means of an ultramicrotome and no further contrast coloration was processed. The sections were mounted on copper grids for Electronic Microscopy examinations without stain. HAADF-STEM images were recorded on a FEG-JEOL2010 microscope operated at 200 kV. The analyses of the nanoparticles were performed by using the HAADF-STEM mode (using a 0.5 nm probe and a camera length of 12 cm) in combination with X-EDS detectors (Oxford Inca...
Energy-200). Thanks to this approach, ferritin and P904 were easy and univocally identified in the sample. In order to obtain representative particle size distributions of the samples, over 200 particles were counted using a series of images recorded with the same magnification (800K). The particle morphology was evaluated by obtaining the line profile of the HAADF signal of 150 particles observed in the same series of images.