High resolution microscopy: new physical approaches for life and health sciences

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As applications of nanotechnologies for life and health sciences get booming, magnetic nanovectors undergo a considerable development. Such composite structures made from polymer spheres encapsulating magnetic nanoparticles or from a nanoparticular magnetic core surrounded by an organic coverage exhibit a combination of physical, chemical and magnetic properties very appropriate for diagnostic by imaging such as Magnetic Resonance Imaging (MRI), or for therapy: targeted pharmaceutical vectorization, therapeutic hyperthermia... When such vectors exhibit a nanometric size, intravenous injection and easy spread in the body of the patients are allowed, while effects related to the specific surface area are increased.

Most of the time, such nanovectors are administered to the patients in liquid suspensions by intravenous injection. It is thus crucial to characterize both the collective behaviour and the individual structure of the vectors in liquid suspension. On the other hand their interactions with the targeted regions in the body have to be understood. For this purposes, a multiscale approach of the structure and properties of such nanovectors has been developed, with structural studies carried out through innovative developments based on electron microscopies down to subnanometric resolution and correlated with physical properties.

To achieve characterisation of nanovectors in liquid media we have developed the application of Wet-STEM, a new mode in transmission of environmental scanning electron microscopy (ESEM), to image the internal structure of the magnetic nanoparticles in liquid suspension and image calculations by Monte Carlo simulations have shown that a nanometric resolution could be theoretically achieved. By the same technique, stability or tendency to flocculation in suspensions can be evidenced with respect to the collective behavior of different nanovectors.

We have investigated the interactions of the nanoparticles with targeted regions. The biodistribution and biotransformation of the USPIO contrast agents in the tissular and cellular environments were investigated at increasing spatial resolution using different techniques. The biodistribution of a MRI contrast agent grafted with a fluorophore, in ex vivo samples from

atherotic aorta and spleen were revealed by biphoton microscopy with a resolution of a few hundred nanometers, down to macrophage scale. Then preparation of ex vivo samples for transmission electron microscopy (TEM) was adapted from standard protocols especially with respect to staining after inclusion in resin. This way, the first high resolution HR(TEM) images and electron diffraction patterns of crystallized USPIO contrast agents in the aorta or the spleen of an atheromatous mouse were obtained. Combining such structural studies with measurement (using a SQUID setup) of magnetic properties, a longitudinal follow-up of USPIO nanoparticles injected in mice for MRI of the atherotic plaque has been completed for USPIO particles embedded in the aorta and the spleen: the results were interpreted in terms of agglomeration of the particles with a decreasing size depending on time after injection and found consistent with a model of in vitro degradation in acidic environment proposed to mimick the lysosomal metabolism.