Fluorescent organic nanocrystals embedded in silicate nanoparticles as tracers for in-vivo imaging.

In tumor biology, imaging of new blood vessel growth (angiogenesis) associated with a tumor during its development, and of changes of the tumor's vascular structure after therapy, is important in the evaluation and validation of new treatment protocols. Modifications in the vascular parameters, such as density, blood volume and perfusion, should be followed over time on the same animal tumor model (mouse). Two-photon fluorescence microscopy is the only technique that allows in vivo imaging of these vascular changes at the microscopic scale and deep in the tissue.

In two-photon fluorescence, a molecule is excited by the simultaneous absorption of two photons that combine their energy. This nonlinear effect occurs precisely at the focussed crossing point of the two laser beams, allowing 3D scanning imaging. For this fluorescence imaging technique, we have developed brilliant tracer particles combining several properties: high intensity of the two-photon excited fluorescence, biocompatibility, and large size (several tens of nanometers) to avoid their diffusion across the blood vessel walls. For this purpose, we have synthesized core-shell nanoparticles (NPs) made up of fluorescent organic nanocrystals (the cores) embedded in an amorphous organosilicate (the shells).

These core-shell NPs were obtained from sol-gel solutions containing a solvent, a fluorescent organic dye, the silicon alkoxide precursors of the final silicate shells, and a small amount of water for alkoxide hydrolysis. These solutions were sprayed to form microdroplets, and carefully dried in a laminar gas flow to control the formation of silicate crusts at the droplet surfaces. Confined nucleation and growth of organic nanocrystals in the core of the silicate spheres completed this one-step process (see Fig. 1).

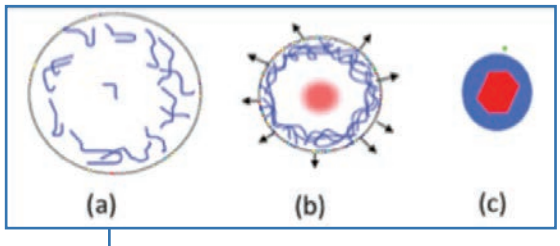

Figure 1 : A droplet drying after sol atomization (a) Initial droplet of homogeneous solution containing the partially hydrolyzed/condensed silicate precursors (blue chains) and the dye dissolved in the organic solvent; (b) Upon drying, a silicate crust is gradually formed at the droplet surface while the confined dye nucleates at the centre; (c) After solvent evaporation, the fluorescent dye is embedded as a single nanocrystal in the core of the organosilicate nanoparticle.

The spray-drying reactor was constructed in our laboratory. The initial sol-gel solutions were drawn into a pneumatic atomizer. The as-produced aerosols, consisting of 1-2 µm droplets, were carried through a laminar air flow, dried gradually at about 150° C in a stainless-steel tube, and collected by electrostatic attraction on exit.

The organic compounds selected for the NP cores were developed by the Chemistry for Optics group of the Ecole Normale Supérieure in Lyon. In the crystalline state they exhibit high 2-photon absorption and high fluorescence emissions in the red and near infrared range, the "biological window" where tissues are transparent. In optimized conditions, the majority (70%) of our core-shell NPs have diameters ranging between 20 nm and 100 nm (see Fig. 2a). The organic cores of the NPs have diameters of order several tenths of nanometers and comprise 10^5-10^7 molecules, yielding fluorescence several orders of magnitude brighter than that of a single molecule. These very brilliant NPs exhibit in general a good photostability, due to their crystallization. Analyzing the crystalline quality of the organic core using Transmission Electron Microscopy in diffraction mode, we obtained well-defined diffraction spots constituting typical patterns for organic single crystals, see the insert in Fig. 2.

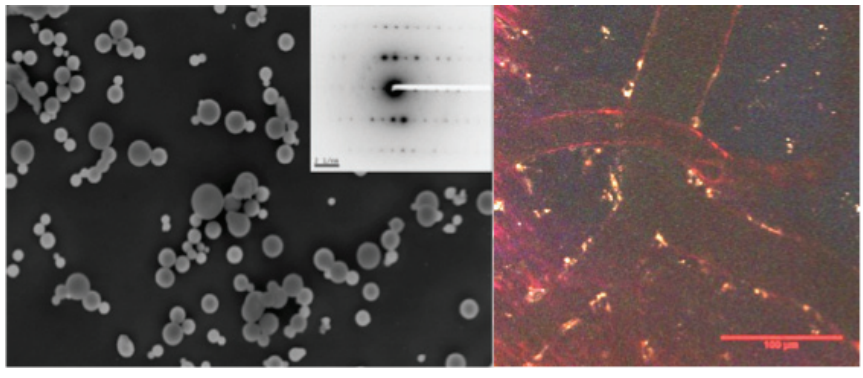

Figure 2 : (a) Scanning Electron Microscope image of nanoparticles prepared from a sprayed sol-gel solution containing CMONS organic dye. Insert in (a): typical electron diffraction pattern showing that the organic cores are single crystals of CMONS. (b) First in vivo, two-photon fluorescence angiography of the microvasculature of the brain of a mouse.

The composition of the silicate shells, which are fully biocompatible and transparent in the visible and near IR, was optimized in a collaboration with the C. Gerhardt Institute, Montpellier University. They allow adjusting the hydrophilicity of the NPs and their subsequent "functionalization" by grafting ligands (peptides) on the NP's surfaces.

Using these new types of nanoparticles, we have done a first in vivo, 2-photon, fluorescence angiography (imaging of blood vessels), at the intravital microscopy platform of the Grenoble Institute of Neurosciences. These first images are very promising as we can clearly see the bright tracers lying on the walls of micro-vessels due to electrostatic attractions (Fig. 2b). This attraction is in fact a typical problem and will be overcome by future developments of the nanoparticle functionalization, aimed in particular at in vivo targeting of the tumor vascular endothelial cells.

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FURTHER READING

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