

Biodistribution of Near-Infrared Fluorescent Nanoparticles: an *in vivo* study

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Introduction:

Research in new fluorescent nanoparticles is today an important field for preclinical studies in the optical imaging technique area. Great expectation concerns new fluorescent markers engineered for particular applications (conjugated with antibody or pharmaceutical compounds) or alone to study nano-compound intrinsic behaviour in living organisms. In particular, nanosized fluorescent particles (silica nanoparticles¹ and quantum dots^{2,3}) are promising tools for the development of luminescent probes and markers provided by great brightness and high photostability respect to traditional organic fluorophores. Here we present an *in vivo* study of biodistribution in a small laboratory animal model of silica-core / PEG-shell fluorescent nanoparticles (20-30nm) doped with a CY7 NIR emitting dye ((2-((E)-2-((E)-2-chloro-3-((Z)-2-(3-ethyl-1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene) ethylidene)cyclohex-1-enyl)vinyl)-3-ethyl-1,1-dimethyl-1H-benzo[e]indolium iodide). Silica particles, due to the recognized low toxicity of their chemical composition, could be interesting for future clinical applications.

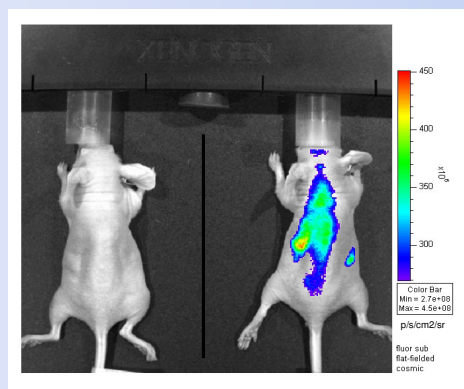


Fig.2: Optical imaging acquisition of control (on the left) and treated (right) nu-nu mice 2h after nanoparticles injection. Exposure time 1 s, binning 8, f/2, excitation filter ICG [710-760 nm], emission filter [810-875 nm].

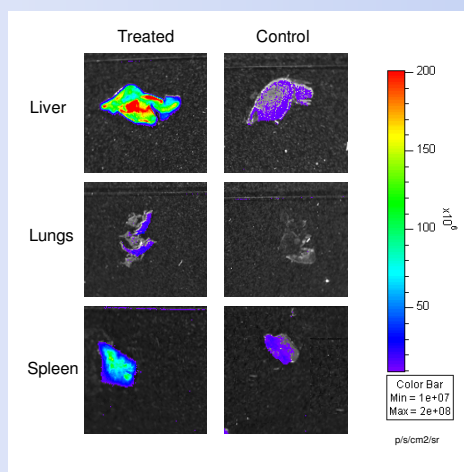


Fig.3: Optical imaging acquisition from extracted liver. On the left we show a treated section and on the right a control. Exposure time 30 s, binning 1, f/1, excitation filter ICG [710-760 nm], emission filter [810-875 nm].

Conclusions:

This preliminary data give us a good characterization of fluorescent characteristics of the silica-core / PEG-shell nanoparticles. They have showed a very good fluorescent efficiency comparable with commercial semiconductors nanocrystals (quantum dots, QDs) actually used in preclinical research. They can be successfully used for *in vivo* applications allowing to follow the biodistribution and tissues accumulation for hours in a small animal model. The very low intrinsic toxicity of the chemical composition encourages the employ of such fluorescent markers for many *in vivo* applications in preclinical research and to investigate the possibility of engineering them with biomolecules for targeting bio-analytical applications.

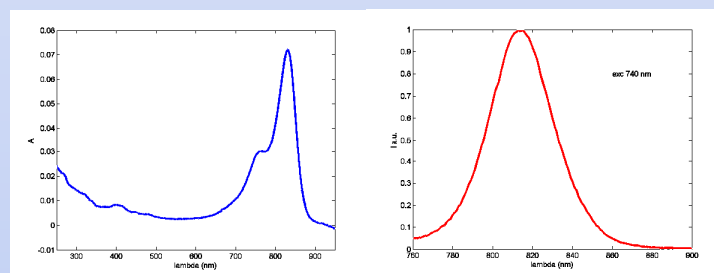


Fig.1: Absorbance (on the left) and emission (on the right) of the near-infrared nanoparticles

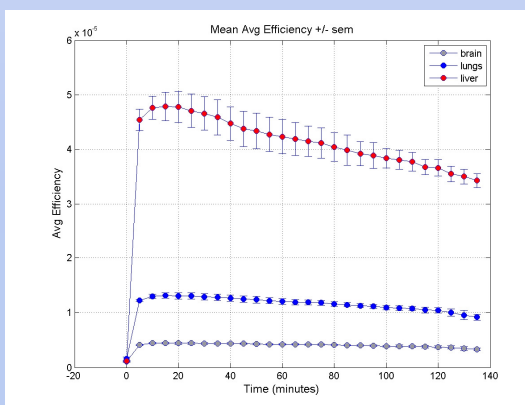


Fig.4: Average efficiency of fluorescence measured on three ROIs corresponding to brain, lung and liver versus time. Every value represents the mean from the treated group.

Methods:

Silica fluorescent nanoparticles biodistribution was studied. We have observed with Optical Imager the kinetics of biodistribution and tissue accumulation during three hours immediately after fluorescent tracer administration (10 μ l/g body weight, i.v.), in gas anaesthetized mice. Optical images were acquired with IVIS200 Series (Xenogen Corporation, Alameda USA). Data were extracted using Living Image 2.6 software.

Silica nanoparticles, with an emission peak around 810 nm, were excited with ICG excitation filter (710-760 nm) and the fluorescent emission acquired with ICG emission filter (810-875 nm).

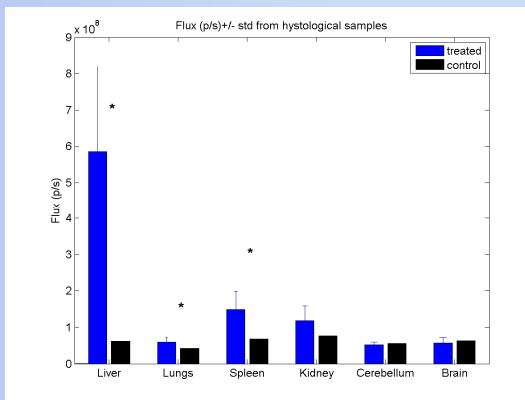


Fig.5: Histogram representing the emitted flux from surgically extracted organs and acquired with optical imaging. The asterisk over the columns indicated the significant differences among treated and control samples ($p < 0.05$)

References:

- Burns A., Ow H., Wiesner U., *Chem. Soc. Rev.*, 2006, 35, 1028-1042; Lin Wang, Wenjun Zhao, Weihong Tan *Nano Res.*, 2008, 1, 99-115;
- Young-wook Jun, Jae-Hyun Lee, Jinwoo Cheon, *Angew. Chem. Int. Ed.*, 2008, 47, 5122 - 5135
- Resch-Genger U., Grabolle M., Cavaliere-Jaricot S., Nitschke R., Nann T., *Nature Methods*, 2008, 5, 763-775;
- Igor L. Medintz, H. Tetsuo Uyeda, Ellen R. Goldman, Hedi Mattoussi, *Nature Materials*, 2005, 4, 435 - 446
- Masotti A., Vicennati P.; Boschi F.; Calderan L.; Sbarbati A.; Ortaggi G. A Novel Near-Infrared Indocyanine Dye-Polyethylenimine Conjugate Allows DNA Delivery Imaging *in vivo*. *Bioconjug Chem.* 2008 May 21;19(5):983-987