An in vivo study of Quantum Dots tissue accumulation.

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Introduction

Nanotechnology represents a new frontier for the science progress and there are great expectations in relation to diagnostic and therapeutic envelopes [1]. Understanding of biological processes on the nanoscale level is an important driving force behind development of nanotechnology. And nanoparticles could be exploited not only like an incredible instrument for scientific research but also could be used to study behaviour of nanoparticles themselves because they are a typical product of the industrial processes and few is known about in vivo toxicology of nanoparticles [2].

In the present work we have used nanoparticles with optical properties, Quantum Dots (QDs) are interesting for the molecular, cellular and *in vivo* small-animal imaging [3]. The first studies conducted on QDs in vivo kinetics by using whole animal fluorescent imaging provides a qualitative assessment of surface-chemistry-dependent kinetics of these nanoparticles [4; 5]. It was very difficult to quantifying and localizing the uptake and the in vivo distribution, clearance, metabolism and toxicity [6; 7]. Fluorescent imaging has shown anatomical districts involved in QDs clearance. However measurements of tracking and quantification of QDs is limited by tissue absorption of light, which impairs excitation of QDs in deeper lying tissues, and decreases fluorescent light penetration from deeper structures from the surface of animals [8; 9].

We try to overcome the limits of tissue absorption of light Using particular characteristics of QDs excitation and emission, exploiting optical properties of tissues and implementing Optical Imager (VivoVision System, IVIS200).

The aim of the study is to set a fully quantitative method to monitor nanoparticles in vivo and to study their bio-distribution in living animals. Physiological data (concerning liver, brain and lung the most probably targets of toxicity) are extrapolated by optical parameters.

Materials and Methods

For all experiments we have used a VivoVision Systems, IVIS® 200 Series,, Xenogen (Xenogen Corporation, Alameda USA), constituted by a camera sensor back thinned, back illuminated grade CCD 1 (2.7 x 2.7cm, -90°C), with a minimal image pixel resolution of 20µm (pixel dimension 13.5µm, imaging pixels 2048 x 2048), quantum efficiency >85% between 500 and 700nm, and > 30% between 400 and 900nm.

Images will be acquired and analyzed with Living Image 2.6 software, Living Image 3D software (Xenogen Corporation, Alameda USA) and Matlab 7.1. Data was analyzed with ROI (region of interest) method.

For all experiments we have used fluorescent modality, with the acquisition set of excitation filters: GFP (445-490nm), DsRed (500-550nm), Cy5.5 (615-665nm), ICG background (665-695nm), ICG (710-760nm); and the emission filter ICG (810-875nm). A 150W Quartz halogen 3250° Kelvin lamp at high intensity; binning factor = 8; pixel resolution = 0.125x0.125; field of view = 12.8cm; exposition time = 1sec; opening of diaphragm (f/stop) = 2.

Acquisition protocol was about a first pre acquisition and 30 post tracers i.v. administration acquisitions.

We have used female athymic nude-nude mice, 3-4 weeks-old; two dosages (1:5 and 1:10 dilution of QDs800 commercial solution /0.01ml/1g body weight, i.v.) (n=6 for each group) we have observed the bio-distribution during three hours immediately after tracers administration, and n=3 for each dosage group to study time of elimination and tissue accumulation after 24h and 1 week from i.v. administration.

Tracers that we have used are nanoparticles about 10-15nm, semiconductors (Qtracker® 800 non-targeted quantum dots, InvitrogenTM Milan, Italy), constituted by a core of CdSe, a shell of ZnS and an amphiphilic inner coating covalently modified with a functionalized polyethylene glycol (PEG) outer coating.

Mice were gas anesthetized (isoflurane, Forane® Abbot s.p.a., Latina Italy) with XGI-8 Gas Anesthesia System (Xenogen Corporation, Alameda USA) and they are placed in a dorsal position on the stage, heated to 37°C.

References

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Liver

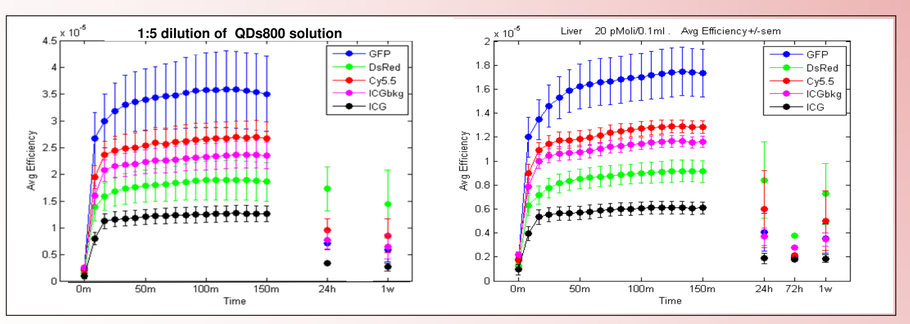

Fig. 1. Average efficiency about kinetic of QDs800 accumulation on cerebral region: a 3h continuous acquisition immediately after i.v. administration; 24 hours and 1 weeks after i.v. administration. Excitation filters used: GFP, DsRed, Cy5.5, ICGbkg e ICG. Emission filter used: ICG

Lungs

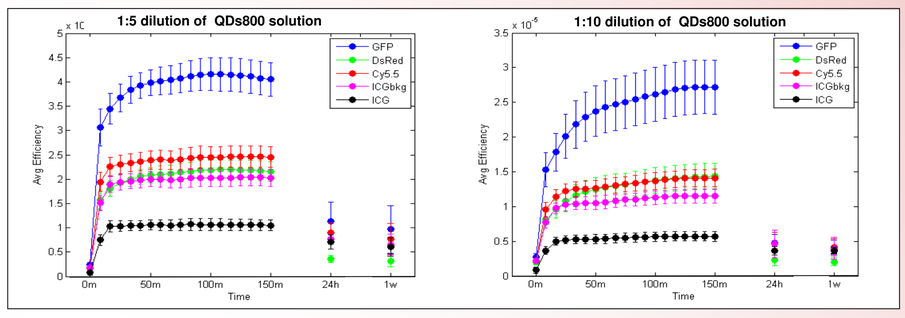

Fig. 2. Average efficiency about kinetic of QDs800 accumulation on pulmonary region: a 3h continuous acquisition immediately after i.v. administration; 24 hours and 1 weeks after i.v. administration. Excitation filters used: GFP, DsRed, Cy5.5, ICGbkg e ICG. Emission filter used: ICG

Brain

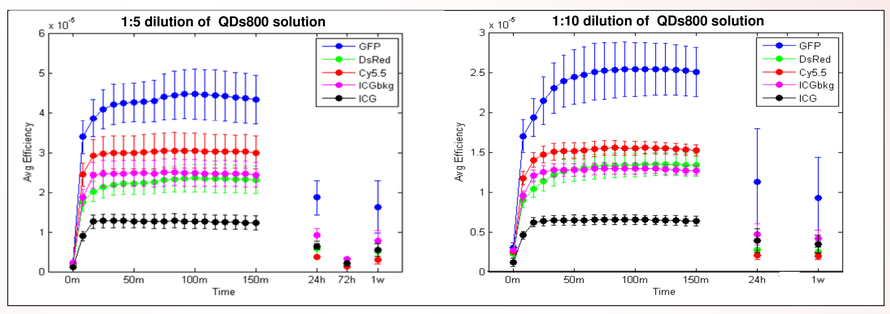

Fig. 3. Average efficiency about kinetic of QDs800 accumulation on hepatic region: a 3h continuous acquisition immediately after i.v. administration; 24 hours and 1 weeks after i.v. administration. Excitation filters used: GFP, DsRed, Cy5.5, ICGbkg e ICG. Emission filter used: ICG

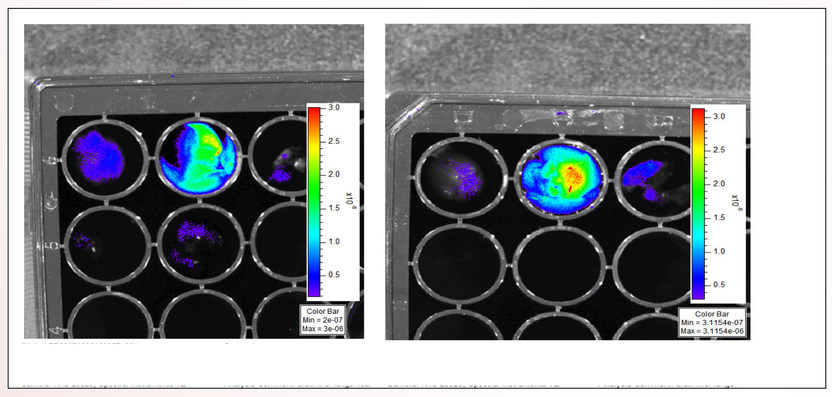

Fig. 4 Ex-vivo acquisition: 1:5 dilution of QDs800 solution /0.01ml/1g body weight, i.v; A. brain; B. liver; C. lungs.

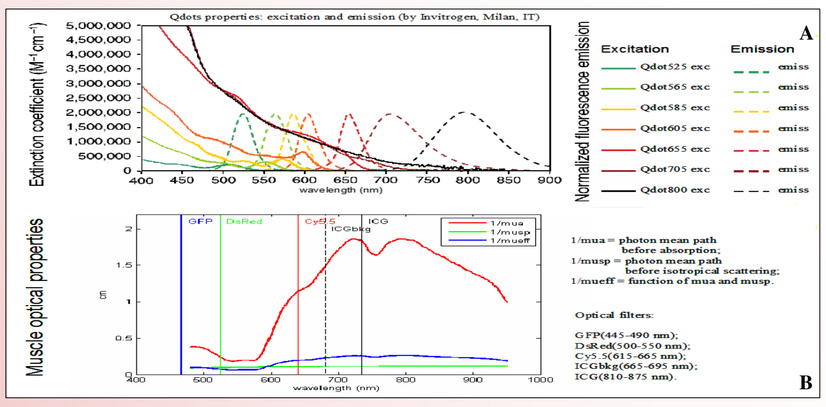

Fig. 5.A. Streptavidine conjugated QDs800 excitation and emission spectri (Invitrogen, Milan Italy) **B.** 1/µa , 1/µs', 1/ µeff path values of muscle in relationship of wavelength from 480 to 950 nm. Wavelengths intervals used in excitation filters (GFP, DsRed, Cy5.5, ICGbkg e ICG) and in emission filter (ICG em.) are marked with different colors.

Results

Fluorescent images of nude mice were acquired with the excitation/emission filters setup. To apply this method three excitation filters (Cy5.5, ICGbkg and ICG) were used to excite QDs800 accumulated in the organs and one emission filter (ICG) to collect the emitted radiation. In muscle the excitation filters select photons with 1.10, 1.48 and 1.77 cm mean free paths before absorption respectively. Still in muscle photons emitted from QDs800 and passing the ICG emission filter have 1.67 cm of mean free path before absorption.

Only pixels belonging to the animal body were considered. For each filter ICGbkg and ICG three intermediate values between the limits (dorsal and ventral surfaces) were identified to form, with the limits, five equally spaced points equally spaced among them. These points were considered the seeds for a classification process of the fluorescence images. The number of seeds was chosen arbitrarily. The average radiance was measured pixel by pixel and normalized to the average radiance measured with the Cy5.5 filter. All the pixels were ranged with a classification process with five classes and every class corresponds to a seed (Fig. 6).

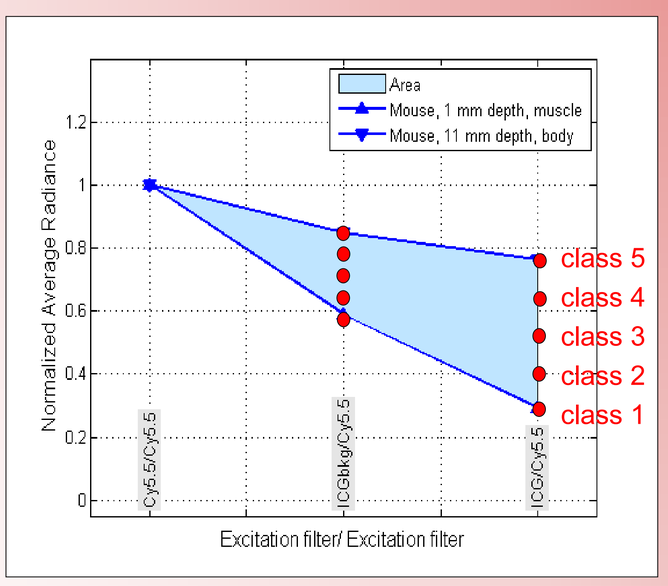

Fig. 6. The average radiance was measured pixel by pixel and normalized to the average radiance measured with the Cy5.5 filter. All the pixels were ranged with a classification process with five classes and every class corresponds to a seed.

The result of classification process applied to one image is shown in Fig.7. The colours indicate five classes. To each class is associated the light path done by the photons before escaping from the animal surface: class 1 = 1 mm, class 2 = 3.5 mm, class 3 = 6 mm, class 4 = 8.5 mm and class 5 = 11 mm.

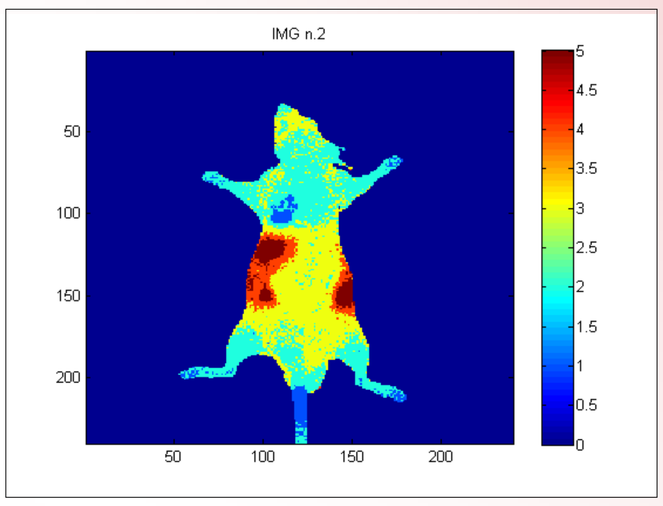

Fig. 7. The colours indicate five classes. To each class is associated the light path done by the photons before escaping from the animal surface: class 1 = 1 mm, class 2 = 3.5 mm, class 3 = 6 mm, class 4 = 8.5 mm and class 5 = 11 mm.

Conclusion

Optical properties of muscle tissue, referent value, are described in Fig. 5B (Living Image 3D software, Xenogen Corporation, Alameda USA). To apply this method three excitation filters (Cy5.5, ICGbkg and ICG) were used to excite QDs800 accumulated in the organs and one emission filter (ICG) to collect the emitted radiation. Using muscle tissue optical properties, the excitation filters (Cy5.5, ICGbkg and ICG) select photons with 1.10, 1.48 and 1.77 cm mean free paths before absorption respectively. Still using muscle tissue optical properties photons emitted from QDs800 and passing the ICG emission filter have 1.67 cm of mean free path before absorption. Wavelengths selected by GFP and DsRed excitation filters have a lower penetration depth (less than 0.5 cm). Only nanoparticles in the superficial layer can be stimulated by them (Fig 5B). The higher average efficiency values in Cy5.5 , ICGbkg and ICG than DsRed excitation filters suggests that Cy5.5, ICGbkg and ICG filters select photons with wavelengths that can penetrate tissue with a depth useful to excite QDs800 in the hepatic region (Fig 4B). This consideration also suggests that Cy5.5, ICGbkg and ICG filters select photons with wavelengths that can penetrate tissue with a depth useful to excite QDs800 in the cerebral and pulmonary regions. This interpretation is encouraged by average efficiency of signal observed in brain, liver and lungs explanted from two animals administered with 1:10 dilution of QDs800 solution (Fig 4) and by preliminar Trasmission Electron Microscopy data here not reported. The continuous increase in the average efficiency values using filter set, during the observed first three hours, encourage the interpretation that the respectively wavelengths are able to excite the nanoparticles in the liver region representing the continuous hepatic build up. Figure 1, 2 and 3 show the first three hours immediately after QDs administration and also the average efficiency on two time points of 24h and 1 week after i.v. administration.

These preliminary data suggest that we could construct an "accumulation map" of QDs800 in time. Besides using selected excitation/emission filters we could obtain a sort of level map of the different tissues in relation to their depth and optical characteristics.