Effect of Ligand Nature on the Kinetics and the Bio-distribution of Quantum Dots in Mice

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ABSTRACT

The meeting of nano-materials with biology has produced a new generation of technologies that can profoundly impact biomedical research. The NIR emitting window (650-900 nm) is appealing for *in vivo* optical imaging because of the low tissue absorption and scattering in this wavelength range. The design of high-quality NIR-emitting quantum dots, with outstanding optical properties in comparison to organic dyes, should therefore lead to novel contrast agents with improved performance. Quantum dot growth is controlled by the coordination of hydrophobic ligands. Hence, they have to be transferred in water and conveniently coated before their use *in vivo*. Several coating strategies are developed. In this paper we report the coating of commercial ITK705-amino particles with methoxy-terminated PEG of different chain length and their *in vivo* behaviour. Fluorescence imaging studies indicate that the speed of first pass extraction of the coated quantum dots towards the liver depends strongly on the PEG length.

Keywords: surface coating, *in vivo* fluorescence imaging, PEG length, speed of extraction.

1 INTRODUCTION

Fluorescence is a tool widely employed in biological assays, from the molecular and cellular level, up to, more recently, the small animal or human level [1,2]. Current organic dyes suffer from many limitations including narrow excitation spectra, broad emission spectra, and photo-bleaching. Fluorescent semi-conducting nano-crystals, also known as quantum dots (QDs), display improved optical properties and are used for biological imaging in both *in vitro* [3–6] and *in vivo* conditions [7–10]. For biological imaging, the main requirements for the QDs are water dispersibility, biocompatibility, chemical stability and robust optical properties. Chemical coatings are essential for meeting these requirements and numerous modifications of the QD surface chemistry have been explored, including the attachment of organic layers of poly(ethylene glycol) (PEG) [8–12] and bovine serum albumin [13–15], and biocompatible and chemically functionalizable inorganic shells such as silica [3,5,16,17]. These layers can prevent chemical degradation and leaching of the toxic core materials, improve cyto-compatibility, reduce non-specific binding, and in some cases enhance the optical properties [14,17]. It has been reported that the carboxyl groups on the surface of quantum dots lead to non-specific binding to oligonucleotides [18]. Methoxy-terminated PEG [19] and hydroxyl [20] coatings have been demonstrated to reduce non-specific binding of QDs to cells. A general strategy to reduce non-specific binding of plasma proteins to QDs through PEG coating is presented here. To advance the field of *in vivo* applications of QDs, it is necessary to reduce the reticulo-endothelial system uptake of the particles, which prevent their efficient targeting to other organs. After intravenous administration, plasma opsonines might adsorb onto the nano-particles, which induces their recognition and phagocytose mediated uptake by macrophages. These macrophages are concentrated in the reticulo-endothelial system (liver, spleen, bone marrow) [21]. It has been previously reported that the coating of quantum dots by PEG chains, especially methoxy-terminated PEG_{5000} (5000 g molecular weight), reduces the ratio of the uptake of nano-particles by the reticulo-endothelial system organs (liver, spleen, bone marrow) in mouse animal models [22]. In this paper, the effect of methoxy-terminated PEG length on the kinetics and the bio-distribution of QDs in mice is investigated more thoroughly.

2 EXPERIMENTAL SECTION

2.1 Functionalization of Quantum Dots

Commercial ITK705-amino particles (QD-PEG_{2000}-NH_2) are purchased from Invitrogen. These nanoparticles are 705 nm emitting CdTe/ZnS quantum dots, with a core diameter of ≈ 10-15 nm. These particles are coated by a polymer (with carboxylate groups as ending moieties) and 2,000 Da weight poly-ethylene-glycol (PEG) chains bearing at their extremity primary amino groups, and are supplied at 8 µM in borate buffer (50 mM borate, pH 8.3). These nano-particles can be functionalized in order to obtain nano-particles coated by PEG chains of different lengths. Using

α-methoxy-w-carboxylic acid succinimidyl ester poly(ethylene glycol) (NHS-PEG_X-OCH_3) (X = 750; 2000; 5000; 13000 and 20000) (Iris Biotech GmbH), QD-PEG_Y-OCH_3 (Y = 2750; 4000; 7000; 12000 and 22000) nanoparticles are obtained using the following protocol:

1. The borate buffer in which the QDs are purchased is exchanged against PBS 1X buffer using a Microcon YM100 spin column.
2. To 125 µL of QD-PEG_{2000}-NH_2 particles in PBS 1X, 1 µmol solution of NHS-PEG_X-OCH_3 in anhydrous DMSO is added, and the nano-particles are incubated 30 minutes at room temperature in obscurity.
3. QD-PEG_Y-OCH_3 conjugates are purified using dialysis against PBS 1X with a 25000 MWCO membrane (Spectrum).
4. QD-PEG_Y-OCH_3 conjugates are concentrated and transferred in sterile PBS 1X buffer using Microcon YM100 spin column.
5. The concentration of the solution is determined using visible absorbance measurements.

2.2 Surface and Size Characterization

Gel electrophoresis is employed to investigate the surface charge properties. Agarose gels (1%) are cast in TBE/H_3BO_4 buffers pH=7.1. A 5 μL aliquot of the nanocrystal solution was mixed with 15 μL of phosphate buffer (PBS), and the mixture was briefly vortexed and loaded in the wells. Typical voltage of 100 V is applied over 60 min. The detection of the nanocrystals in the gel is performed by illuminating the gels and recording the fluorescence images with a CCD camera (see paragraph 2.3). Size analysis of all QDs is performed using dynamic light scattering (DLS), thanks to a Malvern Zeta Sizer Nano instrument (NanoZS).

2.3 Imaging System

A home-made system, adapted from the commercially available Aequoria™ system from Hamamatsu, is used. It is made up of a dark box equipped with a mouse-body temperature controller and a gas anesthesia device. The excitation device is composed of 10 LEDS emitting at 633 nm (adapted from the LuxiFlux™ device available from Hamamatsu) and equipped with interference band pass filters (633BP10 nm from Schott) for a light illumination power of 15 µW.cm^{-2}. The filtered fluorescence signal (filter RG665 from Schott) is measured by a cooled CCD Camera (Orca II BT 512 G, Hamamatsu), placed at 160 mm from the imaging field, with an exposure time that can be adjusted from 10 ms to a few seconds. For quantum dot imaging, the typical integration time used is 500 ms, the gain of the camera is set at medium and the binning is 1x1. The Wasabi™ software (Hamamatsu) is used to drive the set-up and for image processing. Outside of the dark box an anaesthetized mouse (Female NMRI nude mice, 5-6 weeks old, purchased from Janvier) is placed on a warm plate (37.5°C) connected to an anesthesia mask (isoflurane/air 1.5%). 200 µL of particles (40 pmol, 200 nM in PBS 1X buffer) are injected intravenously (IV) via the tail vein and the mouse is imaged at each desired time point.

3 RESULTS AND DISCUSSION

3.1 Surface and Size Characterization

Table 1 reports the average hydrodynamic size of ITK705-amino nano-crystals (P0), as well as the hydrodynamic size of QD-PEG_Y-OCH_3 coated particles (P1 - P5). The average sizes of the coated nano-crystals increase by increasing the PEG weight, as expected.

Samples	Hydrodynamic diameter (nm)
ITK705-amino (P0)	21.8±4.1
QD-PEG_{2750}-OCH_3 (P1)	22.8±5.6
QD-PEG_{4000}-OCH_3 (P2)	26.5±5.6
QD-PEG_{7000}-OCH_3 (P3)	32.3±3.7
QD-PEG_{12000}-OCH_3 (P4)	43.3±6.3
QD-PEG_{22000}-OCH_3 (P5)	47.8±3.4

Table 1: Hydrodynamic size analysis of quantum dots before and after the coating step.

The surface charges of the nanoparticles can be probed by gel electrophoresis, since the migration driving force is electrostatic [23]. The migration of nano-particles generally depends on three major parameters: their molecular weight, their size and steric hindrance, their global charge and its eventual screening effect by a chemical coating. A typical migration pattern at pH 7.1 in 1% agarose gel of samples before (P0) and after coating (QD-PEG_Y-OCH_3 where Y=2750 (P1), Y=4000 (P2), Y=7000 (P3), Y=12000 (P4), Y=22000 (P5)) is shown in Figure 1. First, the charge seems to contribute in a major way to the mobility of the particles as we can see in Figure 1 that QDs can migrate towards the positive or negative electrode in a PEG length dependent manner. The fact that their hydrodynamic diameter increases with the length of their PEG coating does not seem to reduce much the QD gel mobility, however, size effects cannot be excluded. ITK705-amino nano-crystals (P0) have negative carboxylate groups due to their inner polymer coating, and positive ammonium groups on the surface, with a global negative charge at pH 7,1. Therefore, they migrate towards the positive electrode (Figure 1). Whenever short PEG chains are grafted onto P0, the ammonium groups are nearly totally replaced by neutral chains, which induces a higher negative global charge and more efficient migration towards the positive electrode (P1). For longer PEG lengths (P2-P5), the number of conjugated polymer chains decreases and the positive charges remains on surface whereas the PEG chains screen the inner carboxylate charges. It results in an overall

positive surface particle and hence a migration towards the negative electrode.

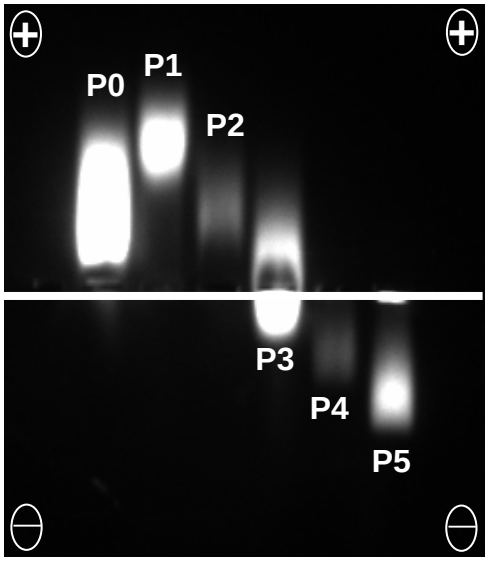

Figure 1: 1% gel electrophoresis of QD-PEG_{2000}-NH_2 and QD-PEG_Y-OCH_3 (P1: Y=2750; P2: Y=4000; P3: Y=7000; P4: Y=12000 and P5: Y=22000) in TBE 1X/H_3BO_4, pH=7.1 at 100 V; the white line marks the loading position.

3.2 In Vivo Imaging of Quantum Dots

The effect of PEG length on the bio-distribution of QD-PEG_Y-OCH_3 in the 24h following tail IV injection is assessed using non-invasive fluorescence imaging in mouse. Immediately after intravenous injection, fluorescence from all QDs is easily seen in the superficial vasculature (Figure 2). Subsequently, QD are seen to deposit in liver, spleen, skin, and bone marrow in a PEG molecular weight dependent manner.

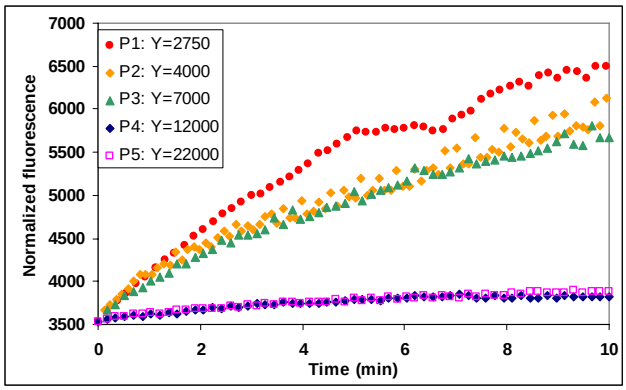

Figure 3: Normalized fluorescence in the liver of mouse injected with the different conjugated quantum dots.

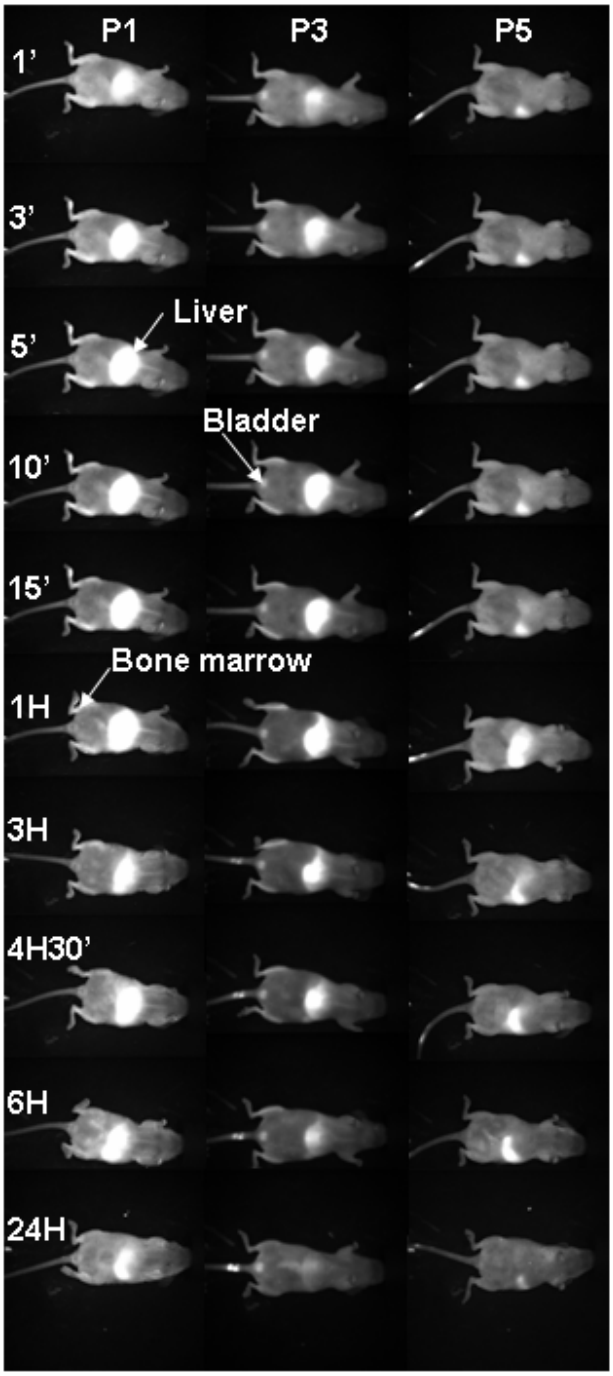

Figure 2: Time-dependent imaging of QD-PEG_Y-OCH_3 (P1: Y=2750; P3: Y=7000 and P5: Y=22000) in live animal over a 24 hour period.

The blood circulation half life is improved when increasing the PEG length. It leads to a slower accumulation in the RES organs, as demonstrated in Figure 3, which represents the normalized fluorescence in the liver for the different conjugated quantum dots during the first 10 minutes following IV injection. The PEG coating is particularly efficient when exceeding 12000 molecular

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weight. Interestingly, the fluorescence in the liver seems to decrease with time starting 3 h post-injection as shown in Figure 2, with again a more pronounced effect for higher PEG length.

4 CONCLUSION

In summary, we demonstrate that the surface coating of quantum dots is a critical parameter in the development of new diagnostic agents. Despite an increase of the hydrodynamic diameter of the particles, by increasing the PEG length we are able to slow down the speed of first pass extraction towards the liver. Hopefully in the future, such coatings will increase the efficiency of organ targeting of QDs, for example in tumour models.

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