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Carbon Dots for Optical Imaging in Vivo

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There has been significant recent interest in the development of highly fluorescent nanomaterials as contrast agents for optical imaging in vivo.[1] The imaging agents should ideally be bright, nontoxic, biocompatible, and stable against photobleaching. Among those extensively studied are ones based on semiconductor quantum dots (QDs) such as CdSe/ZnS.[2] The rationale for the use of QDs over conventional organic dyes is now generally accepted in the literature.[3] There are already successful in vivo imaging demonstrations of QDs on tumor vasculature, tumor-specific membrane antigens, sentinel lymph nodes, and so on.[2,4]

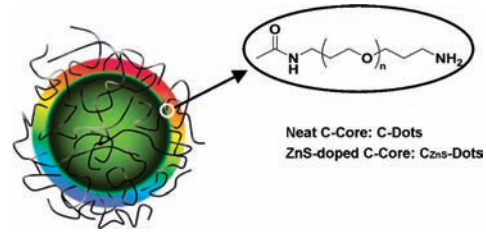
Semiconductor QDs containing cadmium or other heavy metals are unfortunately known for their significant toxicity even at relatively low concentrations,[5,6] which may prove prohibitive to any patient studies. Therefore, the search for benign alternatives has continued. Of particular interest and significance was the recent finding that small carbon nanoparticles could be surface-passivated by organic or biomolecules to become strongly fluorescent.[7] These fluorescent carbon nanoparticles,[7,8] dubbed "carbon dots" (C-Dots, Scheme 1), were found to be physicochemically and photochemically stable and nonblinking. The carbon particle core could also be doped with an inorganic salt such as ZnS before the surface functionalization to significantly enhance the fluorescence brightness (C_{ZnS}-Dots, Scheme 1).[9] These carbon dots have successfully been used for in vitro cell imaging with both one- and two-photon excitations.[7,9,10]

Carbon is hardly considered as an intrinsically toxic element. Available results from the ongoing toxicity evaluation of oligomeric PEG-functionalized C-Dots[7] in mice have suggested no meaningful toxic effects,[11] raising the prospect for in vivo biocompatibility and uses of carbon dots. Here we report the first study of carbon dots for optical imaging in vivo. The results suggest that the carbon dots are not only brightly fluorescent in solution, as reported previously,[7,9] but also well-behaved as contrast agents in live mice.

C-Dots and C_{ZnS}-Dots with PEG diamine, $H_2NCH_2(CH_2CH_2O)_nCH_2CH_2CH_2NH_2$ ($n \approx 35$, PEG_{1500N}), as the surface passivation agent were prepared and characterized as previously reported.[7,9,10] Shown in Figure 1 are representative atomic force microscopy (AFM) and high-resolution transmission electron microscopy (HRTEM) images of the carbon dots. Both samples were readily soluble in water to form stable aqueous solutions suitable for the various injections described below.

For subcutaneous injection, female DBA/1 mice (~25 g) were shaved in the back area surrounding the injection point. Upon injection of a C-Dots solution (30 μg carbon-core equivalent in 30 μL) or a C_{ZnS}-Dots solution (65 μg in 30 μL), the mice were imaged

Scheme 1



in a Lumazone FA in vivo imaging system (MAG Biosystems) with 470 nm (~40 nm fwhm) excitation and 525 nm (~47 nm fwhm) emission filters. As shown in Figure 2, the fluorescence images of the subcutaneously injected mice exhibited bright emissions from C-Dots and C_{ZnS}-Dots. The relatively stronger fluorescence from the latter is consistent with the previously reported solution-phase results.[9] The injected carbon dots in mice diffused relatively slowly, and the fluorescence faded at ~24 h postinjection.

The carbon dots could be excited at longer wavelengths for red fluorescence emission. For the same subcutaneous injection into mice, the imaging results with 545 nm (~29 nm fwhm) excitation and 620 nm (~59 nm) emission filters also exhibited significant fluorescence from both C-Dots and C_{ZnS}-Dots (Figure 2).

The brighter green fluorescence of C_{ZnS}-Dots was used in the imaging to track the migration through lymph vessels. Upon interdermal injection into the front extremity (10 μg in 10 μL), the carbon dots migrated along the arm (Figure 3). Unlike semiconductor quantum

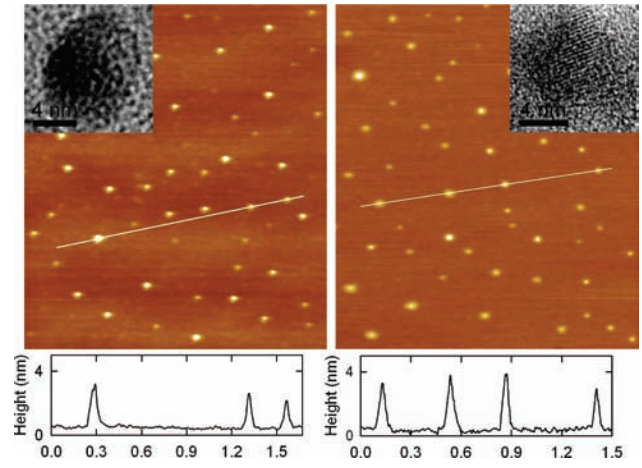


Figure 1. AFM topography images of (left) C-Dots and (right) C_{ZnS}-Dots on mica; the insets show corresponding HRTEM images of individual dots.

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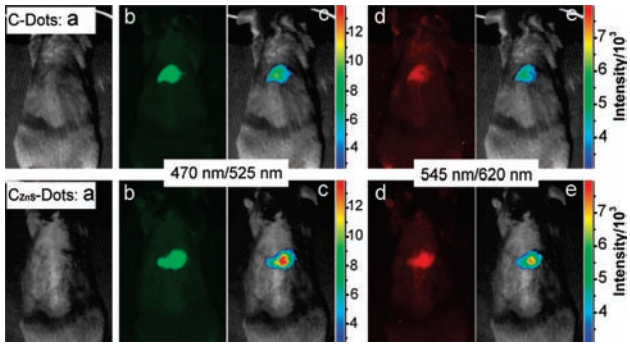

Figure 2. Subcutaneous injection of (top) C-Dots and (bottom) C_{ZnS}-Dots: (a) bright field, (b, d) as-detected fluorescence (excitation/emission wavelengths indicated), and (c, e) color-coded images (ImageJ from NIH).

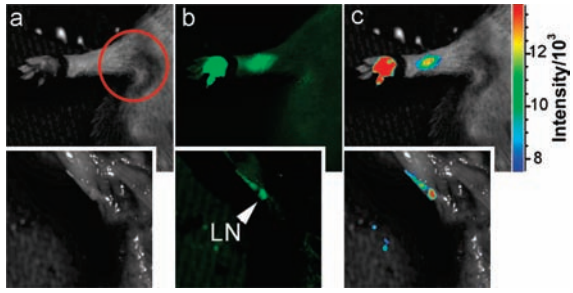

Figure 3. Interdermal injection of C_{ZnS}-Dots: (a) bright field, (b) as-detected fluorescence, and (c) color-coded images. Insets: dissected (in the circled area) axillary lymph node (LN).

dots such as CdSe/ZnS, which can migrate to axillary lymph nodes in minutes,[4c] the observed migration of the carbon dots was slower. This could be due to the small sizes of carbon dots (on the order of 4−5 nm) and/or the surface functionalization by the PEGs, whose protein resistance characteristics might reduce interactions of the carbon dots with lymph cells. The axillary lymph nodes were harvested and dissected at 24 h postinjection and exhibited readily detected fluorescence from the carbon dots (Figure 3).

A C-Dots solution (440 μg in 200 μL) was intravenously injected into mice for whole-body circulation. The abdomen was shaved for fluorescence detection of the dots trapped in organs during the circulation, but only emissions from the bladder area were observed (Figure 4). At ~3 h postinjection, bright fluorescence in the urine became visible in the imaging facility (Figure 4). The results suggest that the intravenously injected carbon dots are primarily excreted via urine, an excretion pathway that has been widely reported in the literature for PEGylated nanoparticles, especially for very small particles like the ones used here.[12]

The organs were harvested at 4 h postintravenous injection for imaging analyses ex vivo. Only the dissected kidneys and liver exhibited meaningful fluorescence from the carbon dots, which was brighter in the former (Figure 4), consistent with the urine excretion pathway. The relatively weak fluorescence in the dissected liver was an indication of a low accumulation level of the carbon dots. While generally significant hepatic uptake of nanoparticles and nanotubes has been widely observed and discussed in many studies,[13] the low accumulation here might again be attributed to the effective surface PEGylation that probably reduced the protein affinity and made the carbon dots stealthy with respect to hepatic uptake.

All of the reported animal experiments were performed at Clemson University by strictly following the IACUC-approved protocols. During the experiments, no animal exhibited any sign of acute toxicological responses.

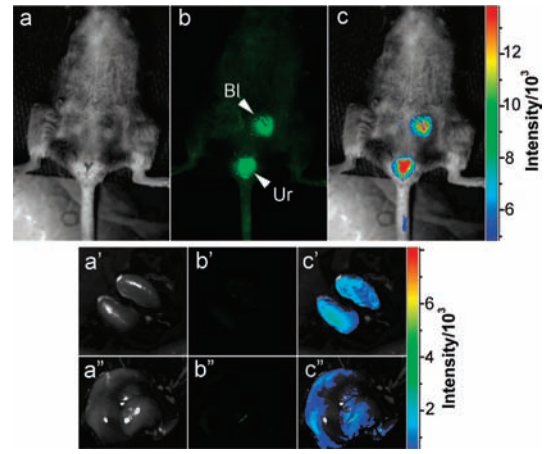

Figure 4. Intravenous injection of C-Dots: (a) bright field, (b) as-detected fluorescence (Bl, bladder; Ur, urine), and (c) color-coded images. The same order is used for the images of the dissected kidneys (a′−c′) and liver (a″−c″).

In summary, the results reported here demonstrate that carbon dots injected in various ways into mice remain strongly fluorescent in vivo, which, coupled with their biocompatibility and nontoxic characteristics, might offer great potential for optical imaging and related biomedical applications.

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Supporting Information Available: Complete ref 7 and additional experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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