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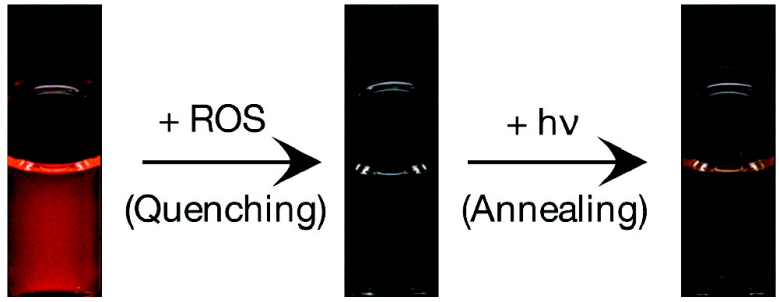

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Oxidative Quenching and Degradation of Polymer-Encapsulated Quantum Dots: New Insights into the Long-Term Fate and Toxicity of Nanocrystals in Vivo

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Semiconductor quantum dots (QDs) are a new class of fluorescent labels under intense research and development for broad applications in molecular, cellular, and in vivo imaging.[1] These nanometer-sized particles have unique functional and structural properties, such as size and composition tunable fluorescence emission, large absorption cross sections, and exceptional brightness and photostability when compared to organic dyes and fluorescent proteins. QDs also provide a versatile nanoscale scaffold for designing multifunctional nanoparticles with both imaging and therapeutic functions. Recent advances have led to the development of bright and stable QD probes for molecular imaging and targeting at high sensitivity and specificity.[2] The in vivo fate and potential toxicity of quantum dots, however, remain poorly understood.[3] In particular, it is not known if polymer-encapsulated QDs would stay intact inside cells and organs or if the QDs could be degraded or removed by certain mechanisms in vivo. This question is of both fundamental and clinical significance because it is likely to determine whether semiconductor nanocrystals could be used for injection into human patients.

Here we report an oxidative mechanism involving hypochlorous acid (HOCl) and hydrogen peroxide (H_2O_2) that leads to fluorescence quenching and chemical degradation of polymer-encapsulated QDs. These molecules are both reactive oxygen species (ROS) produced during cellular metabolism and are important in the pathogenesis of a variety of inflammatory diseases including cancer and atherosclerosis.[4] We have found that HOCl in its neutral form is especially potent (rapid kinetics) in quenching and degrading polymer-encapsulated (poly(acrylic acid) graft dodecylamine, PAA-*g*-DDA) QDs (core−shell CdSe/CdS/ZnS). In comparison with other ROS such as superoxide (O_2^-), hydroxyl radicals (•OH), and peroxynitrite ($ONOO^-$), HOCl and H_2O_2 are unique in their neutral charge and stability under physiologic conditions. Thus, we believe that they are able to diffuse across the polymer coating layer, causing chemical oxidation of sulfur and selenium atoms on the QD surface. This "etching" process generates lattice defects and quenching fluorescence and produces soluble Cd, Zn, S, and Se species. In fact, significant fluorescence quenching occurs before UV−vis absorption changes with as few as 10 HOCl molecules per QD, indicating that fluorescence quenching is caused by localized surface defects. Remarkably, we find that these localized defects can be repaired or "annealed" by photoillumination prior to significant QD dissolution.

Figure 1A shows quenching of QD fluorescence as a function of HOCl to QD molar ratios. Decreases in the photoluminescence (PL) intensity are observed by simple visual inspection at a molar ratio of 100 HOCl per QD (see color fluorescence images of QD samples in vials, Figure 1A, left panel). Quantitative measurements show a detectable decrease in the PL intensity at molar ratios as low as 10 HOCl per QD, with complete quenching at 10 000 HOCl per QD (Figure 1A, right panel). We attribute the rapid loss of QD fluorescence following HOCl exposure to binding of HOCl to chalcogenides on the QD surface. This binding event generates a surface defect site for nonradiative exciton recombination and efficient fluorescence quenching.[5] The physiological concentrations of HOCl are typically 5−25 μM, corresponding to 100 to 500 HOCl molecules per QD (see Supporting Figure 1). For in vivo animal studies, this ratio is likely much higher because the injected QDs are rapidly diluted by blood circulation. QD quenching and degradation are also observed with hydrogen peroxide, but the kinetic rates are much slower (see Supporting Figure 2).

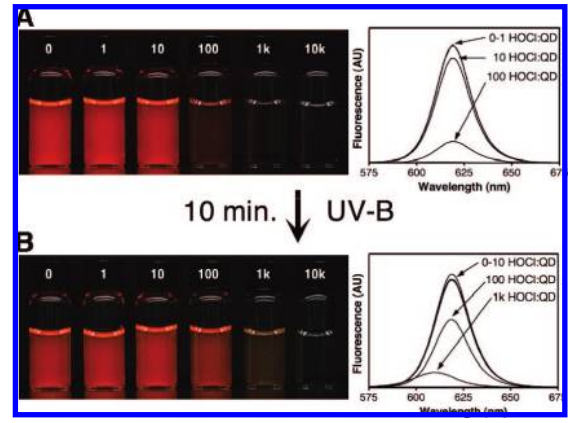

Figure 1. (A) Quenching of QD fluorescence by hypochlorous acid (HOCl) and (B) restoration of QD fluorescence with UV−B light illumination. Shown on the left are fluorescence images of 50 nM polymer (PAA-g-DDA) encapsulated QDs exposed to increasing concentrations of HOCl (ranging from 0 to 10k HOCl molecules per QD) before or after exposure to UV light. Shown on the right are photoluminescence spectra obtained from the HOCl treated QD samples before and after UV light exposure.

Brief exposure to UV light partially restores QD fluorescence, similar to a process called "photoannealing".[6] As shown in Figure 1B, following UV illumination, QDs exposed to 100 and 1000 HOCl per QD become more fluorescent, and in the case of QDs treated with 1000 HOCl per dot, the QD emission also showed a 10-nm blue shift from 619 to 609 nm; the control QD sample (not treated with HOCl) showed no emission peak shift after photoannealing, and no peak shift was observed in any sample before photoannealing. Based on the photoluminescence data of these QDs obtained during synthesis (see Supporting Information), a 10-nm blue shift corresponds to a size decrease of about one monolayer, or a loss of several hundred chalcogenide atoms.

To confirm that HOCl is indeed etching the QDs, we have investigated changes in the QD electronic structure and the reaction byproducts. As shown in Figure 2A, UV−vis optical spectroscopy reveals a decrease in absorbance and a blue shift in the first exciton peak (609 nm). These absorption changes occur at a molar ratio of

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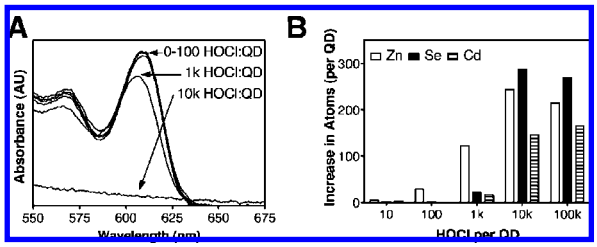

Figure 2. (A) UV−vis absorption spectra and (B) ICP-MS elemental analysis data showing chemical degradation of 50 nM QDs (polymer-encapsulated CdSe/CdS/ZnS) after exposure to hypochlorous acid.

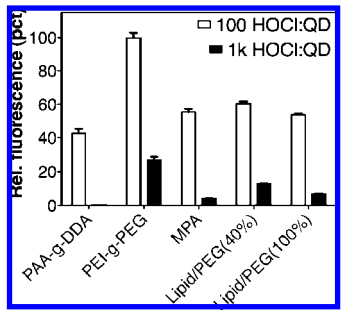

Figure 3. Comparison of fluorescence quenching at two HOCl:QD ratios for different surface coating materials. PAA-g-DDA: poly(acrylic acid) with grafted dodecylamine; PEI-g-PEG: polyethyleneimine with grafted polyethylene glycol (MW 2000); MPA: mercaptopropionic acid; Lipid/PEG(40%) and Lipid/PEG(100%) (see Supporting Information). Error bars show the standard deviations ($n = 4$).

1000 HOCl molecules per QD, the same ratio that also causes a spectral shift in fluorescence after photoannealing. Consistent with previous reports,[6] there are no detectible changes to the UV−vis spectra following photoannealing. These spectral changes are consistent with a decrease in the QD size caused by HOCl etching.

At a molar ratio of 10 000 HOCl per QD, the UV−vis spectra become featureless, indicating severe QD degradation and dissolution. Definitive evidence for QD etching is provided by elemental analysis of the etching solution using inductively coupled plasma-mass spectrometry (ICP-MS). As shown in Figure 2B, the soluble Zn, Cd, and Se species that are generated by etching increase with HOCl concentration (note that S cannot be detected on the quadrupole ICP-MS instrument used). Previous studies have shown that QDs can release constituent materials into the solution.[3b] The QDs used in this study have a core material of CdSe and a shell material of CdS/ZnS. As would be expected for such a structure, ICP-MS analysis detects an increase in free Zn at lower concentrations of HOCl exposure, consistent with etching of the shell material, and increases in free Cd and Se at higher HOCl concentrations, consistent with etching of the core material. Significantly, the ICP-MS data are in agreement with the estimated QD chemical composition ($Cd_{0.65}Zn_{0.35}Se_{0.35}S_{0.65}$).

To further examine how surface encapsulation materials might affect QD quenching and degradation, we have investigated a variety of surface coatings,[7] including: (i) ligand exchanged molecules (mercaptopropionic acid, MPA; and polyethyleneimine graft polyethylene glycol-2000, PEI-*g*-PEG_4) interacting with QDs through sulfur (MPA) or amine (PEI-*g*-PEG_4) groups; (ii) lipid/PEG with 40% or 100% PEGylation coverage,[8] and (iii) alkylated amphiphilic polymers (poly(acrylic acid) graft dodecylamine, PAA-*g*-DDA). As shown in Figure 3, oxidative fluorescence quenching is observed for all these coating materials. It is interesting, however, that the ligand-exchanged coatings have greater fluorescence remaining than the PAA-*g*-DDA polymer used for encapsulation. We believe that the functional groups present are reacting with HOCl before it can react with the QD surface; that is, these coordinating ligands can "buffer" the QD local environment against HOCl oxidation. In particular, we find that the PEI-*g*-PEG_4 coating, having free amines that are HOCl reactive, is resistant to HOCl quenching until its buffering limit is reached (nearly 1000 HOCl molecules per QD). In a similar fashion, free reducing agents such as mercaptoethanol are able to partially protect QDs from oxidative quenching by scavenging HOCl molecules (see Supporting Figure 3).

In conclusion, we have reported an oxidative mechanism involving hydrochlorous acid and hydrogen peroxide that results in fluorescence quenching and chemical degradation of polymer-encapsulated QDs. For implications regarding the long-term fate and potential toxicity of semiconductor nanocrystals, phagocytic cells (e.g., neutrophils and monocytes) are capable of generating HOCl at concentrations estimated to exceed 20 μM.[9] These phagocytes as well as macrophages are also known to accumulate quantum dots and other nanoparticles in vivo.[10]

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Supporting Information Available: Experimental methods, QD synthesis data, and Supporting Figures 1−3. This information is available free of charge via the Internet at http://pubs.acs.org.

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