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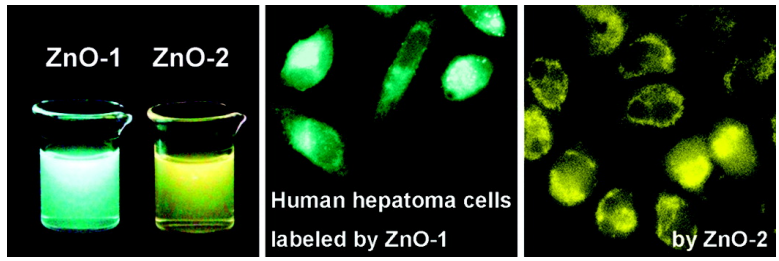

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Stable Aqueous ZnO@Polymer Core−Shell Nanoparticles with Tunable Photoluminescence and Their Application in Cell Imaging

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Photoluminescent semiconductor quantum dots (QDs) with potential application as biological labels have received intensive attention during the past decade.[1] These QDs possess several advantages in comparison with the fluorescent organic species: they are stable under ultraviolet (UV) light, their emission peaks are narrow and symmetric, their emission wavelength can be tuned by varying particle diameter, different colors can be observed under a single excitation light, and more importantly, the QDs are much cheaper than fluorescent organics. However, the typical QDs based on CdSe and CdTe species are vital to the biological systems.[2] Although various protections employing ZnS, polymers, and other nontoxic shells have been developed recently,[3] the leakage of Cd ions through the shell defect, the decomposition of nanoparticles by oxygen, and the radicals derived from light irradiation will destroy the biological systems.[4] Furthermore, producing and employing Cd-related compounds are harmful to human health and eventually damage the environment. Therefore, searching for nontoxic substitutes is an actual challenge in this field.

ZnO QD is a promising candidate because it is a cheap nontoxic photoluminescent semiconductor. Although such merits were found 20 years ago,[5] ZnO has not been tried as a biological label so far. ZnO QDs derived by traditional sol−gel approaches have several drawbacks: low quantum yield (QY, usually below 10%), unstable emission peak, broad photoluminescence (PL) band, and poor water stability. These drawbacks are ascribed to the insufficient protection of the previous synthetic methods and the ZnO luminescent mechanism which depends on its surface vacancies.[6] However, significant improvements have been made in the past few years. ZnO PL emission has been successfully tuned from blue to yellow,[7] its QY has been improved above 30% (even to 85%),[7,8] and new luminescent mechanisms[9,10] related to surface groups have been suggested. The latest report[10] demonstrated that ZnO QDs could exist stably in aqueous solutions if a proper synthetic route was chosen. But the obtained ZnO QDs only emitted ca. 430 nm light and they were not suitable for biological labeling because most cells and tissues also appear blue under UV light. Hence, ZnO QDs with green and yellow emission have better prospects in biological labeling. In this paper, we show a novel method to prepare highly efficient green and yellow emitting ZnO@polymer core−shell nanoparticles that are stable in aqueous solutions. These QDs are safe for living cells when their concentrations are below 0.2 mg/mL and are able to penetrate into the living cells labeling the cytoplasm.

Experimentally, 0.27 g of 2,2′-azobisisobutyronitrile (AIBN) and 15 mL of poly(ethylene glycol) methyl ether methacrylate (PEGMEMA, M_n = 475) were dissolved in 100 mL of 0.1 M zinc methacrylate ethanol solution. The solution was stirred and heated at 80 °C for 2 min. Then 0.27 g of AIBN and 100 mL of 0.14 M LiOH ethanol solution (or 1 mL of 10 M NaOH aqueous solution) were added into the reaction system and refluxed for 1 h. After cooling to room temperature, the solution was dialyzed against deionized water for 3 days. The obtained QDs were designated as ZnO-1 (derived from LiOH) and ZnO-2 (derived from NaOH), respectively. The PL spectra were recorded by a Varian Cary Eclipse spectrofluorometer. The high resolution transmission electron microscope (HRTEM) images and the electron diffraction (ED) patterns were obtained by a JEM-2010 transmission electron microscope operating at 200 kV. Human hepatoma cells (QGY 7763) were cultured on a slide chip for 1 day, and then treated with 0.1 mg/mL of ZnO-1 or ZnO-2 solution in serum-free medium for 4 h followed by rinsing three times with a phosphate buffer solution. The differential interference contrast (DIC) pictures and the fluorescence images (excited by 350 nm light) of these cells were taken by an Olympus IX2-DSU disk scanning confocal microscope.

The conventional ZnO QDs are unstable in water because water will exchange the organic protecting groups on the ZnO surface, destroy the luminescent centers, and render ZnO QDs aggregates.[11] To obtain water-stable ZnO QDs, our strategy is to synthesize and protect ZnO QDs in anhydrous solvent and then to transfer them into aqueous solution. After polymerization between ZnO-MAA QDs and PEGMEMA monomers, the final product has a copolymer shell with the internal hydrophobic polymethacrylate layer and the external hydrophilic poly(ethylene glycol) methyl ether groups. IR analyses prove that the polymer shells are connected to ZnO cores through covalent bonds.[12] The average molecular weight of the polymer shell is about 4000 as confirmed by GPC measurements, and the ZnO content in the core−shell nanoparticles is about 8 wt % as determined by TG and ICP techniques. Such hydrophobic−hydrophilic copolymer shells make the whole nanoparticles miscible with water on one hand and, on the other, isolate ZnO cores from water. As a result, our ZnO@polymer core−shell nanoparticles exhibit very stable PL in aqueous solutions.

Figure 1 lists the PL spectra and the QY evolution for ZnO-1 and ZnO-2 aqueous solutions. The ZnO-1green emission at about 520 nm and the ZnO-2 yellow emission at about 550 nm are typical ZnO vacancy luminescence. Their absorption onsets locate around 3.4 eV, which is also typical for ZnO band gap. It is known that ZnO emission wavelength and QY depend on both the particle size and the particle surface state. Since ZnO-1 and ZnO-2 have similar composition and structure, the differences in their emission wavelength and QY can be ascribed to the particle size variation. NaOH aqueous solution hydrolyzes the zinc salt more quickly than LiOH in ethanol. Thus ZnO-2 grows faster and larger than ZnO-1. As a result, ZnO-1 solution exhibits a QY over 50% for several

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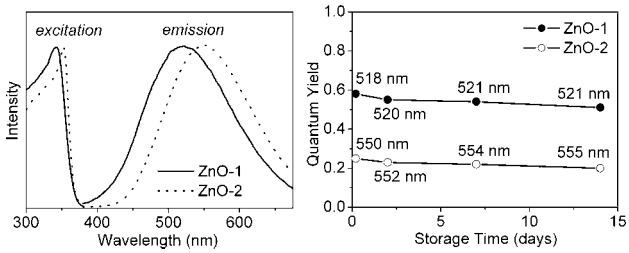

Figure 1. PL spectra for ZnO-1 and ZnO-2 aqueous solutions (left) and their quantum yield evolution at room temperature (right). In the left part, ZnO-1 and ZnO-2 are excited by 340 and 350 nm light respectively to record their PL emission, while their excitation spectra are obtained by setting 520 and 550 nm as the emission maxima, respectively. In the right part, the emission peak wavelengths of ZnO-1 and ZnO-2 are marked near the QY data points, respectively.

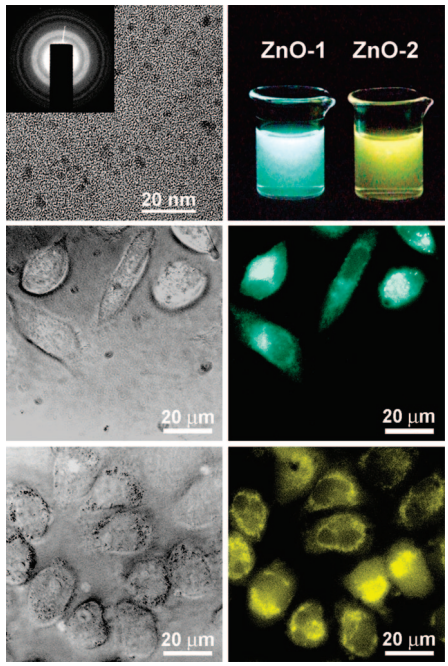

Figure 2. The upper part are the HRTEM image of ZnO-1 with the inset of ED pattern (left) and the aqueous solutions of ZnO-1 and ZnO-2 under a UV light (right); the middle part are the DIC picture (left) and the fluorescent image (right) of the cells labeled by ZnO-1; the lower part are the DIC picture (left) and the fluorescent image (right) of the cells labeled by ZnO-2.

weeks while ZnO-2 has a QY of about 20%. In comparison with other Cd-free QDs,[2b,c] both ZnO-1 and ZnO-2 possess considerably higher QY.

In accordance to the excitation peak positions and the UV absorbance onsets, the HRTEM images indicate that ZnO-1 has an average diameter of about 3 nm and ZnO-2 is about 4 nm. Although the exact diameters and crystal lattice of these ZnO QDs cannot be seen clearly by HRTEM because the organic component exceeds 90 wt % in each sample, the ED pattern in Figure 2 proves that ZnO cores have a wurtzite structure.

Under UV light, ZnO-1 looks green while ZnO-2 appears yellow. The high QY of their aqueous solutions ensures their application in cell imaging. The cytotoxicity tests show that when the concentrations of ZnO-1 and ZnO-2 are below 0.2 mg/mL, more than 90% of human hepatoma cells survive. The DIC pictures and fluorescent images are taken under a ZnO@polymer concentration of 0.1 mg/mL, as shown in Figure 2. It is very clear that the ZnO-1 and ZnO-2 QDs penetrated into the living cells and exhibit bright fluorescence. These QDs are located in the cytoplasm and appear not to exist in the karyons. Under continuous irradiation of UV light, the ZnO luminescence is very stable during cell culture, and the cells are alive within 45 min. Since taking a picture by the confocal microscope only costs a few seconds, we can obtain enough photos for a practical study in which the UV light irradiation is covered in the intervals of cell imaging.

In summary, stable aqueous ZnO@polymer core−shell nanoparticles with tunable PL emission and high QY are successfully prepared through a simple sol−gel method. These ZnO QDs are almost nontoxic for the human cells and they can be used as fluorescent probes in vitro. Our present work proves that ZnO QDs, as a type of safe and cheap luminescent labels, have an inspiring prospect in the biological applications.

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Supporting Information Available: Scheme of synthetic route, IR, and TG results for the final products, GPC curve for the polymer shell, UV−vis absorption spectra, PL spectra evolution, data for QY calculations, the cytotoxicity testing results, and the ZnO luminescent stability study during cell culture under UV light. This material is available free of charge via the Internet at http://pubs.acs.org.

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