Fluorescent Probes

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Highly Luminescent Water-Dispersible Silicon Nanowires for Long-Term Immunofluorescent Cellular Imaging**

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Hybrid nanomaterials that incorporate different classes of nanostructures, such as nanoparticles, nanowires, and nanotubes, have been intensively studied, both in theory and application.[1,2] Compared to a unitary nanostructure, nanohybrids feature many attractive merits. For example, desirable properties can be rationally designed by tailoring the architecture of nanohybrids, which are significant for fundamental understanding of the relationship between physical/chemical properties and nanostructures.[1] Moreover, multifunctional nanohybrids can be prepared by combinations of nanostructures of with unique merits to address various applications.[2] Silicon nanostructures have shown great promise for various applications owing to their unique optical/electronic/catalytic properties, convenient surface functionality, and compatibility with silicon technologies, etc.[3] For example, silicon-based nanohybrids made of silicon nanowires (SiNWs) decorated with noble-metal nanoparticles (for example Au, Ag, Pt) have been recently developed and utilized for solar cells, catalysts, and chemical/biological sensors.[3d,4]

Herein, we present a novel kind of multicolor quantum-dot (QD)-decorated SiNW, which are directly prepared in the aqueous phase by a facile one-pot strategy assisted by microwave irradiation. Remarkably, the SiNWs exhibit strong fluorescence (quantum yield of ca. 30–35 %) with controllable emission wavelengths of 520–620 nm and excellent photostability, and they are well-suited to long-term and real-time immunofluorescent cellular imaging. Figure 1 a presents a representation of our synthetic strategy. In brief, SiNWs are first modified by 3-mercaptopropionic acid (MPA) molecules (A→B) at 100–160 °C by microwave-induced heating through the reaction of MPA molecules with the SiNW surface via the carbonyl group by forming a Si–O bond upon exposure to external energy (for example light irradiation, high temperature).[5] Together with MPA molecules, the resultant MPA-modified SiNWs, acting as ligands, react further with Cd^{2+} and Te^{2-} ions to form crystal nuclei (B→C).[6] The Ostwald ripening stage takes place during prolonged reaction (C→D),[6a,b] leading to in situ growth of QDs

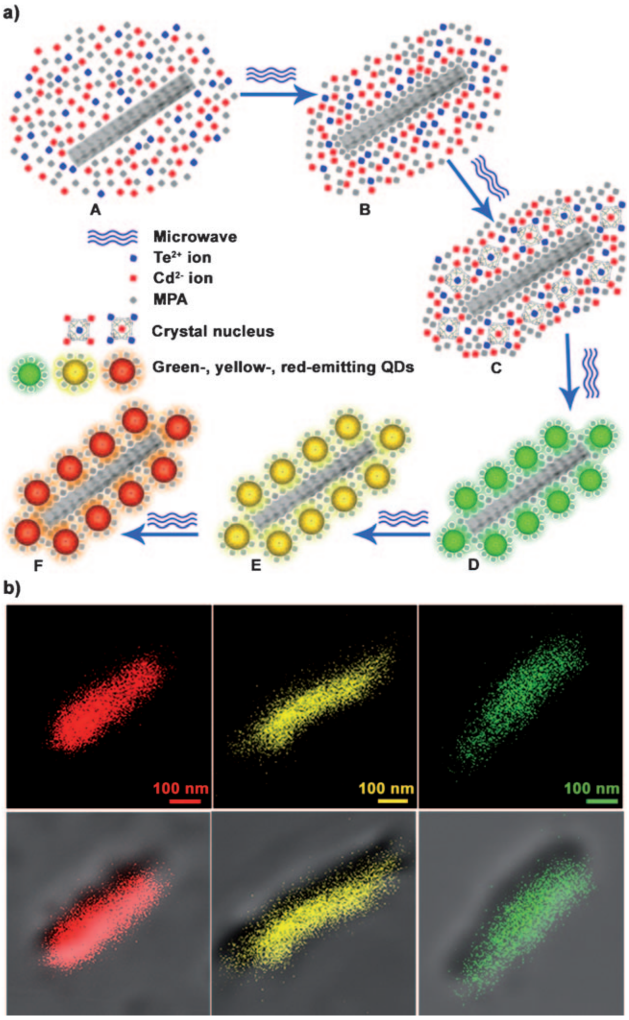

Figure 1. a) The synthesis of multicolor QD-decorated SiNWs (see text for details; MPA = 3-mercaptopropionic acid). b) Fluorescence images of the green, yellow, and red SiNWs obtained with a laser-scanning confocal microscope (LSCM) ($\lambda_{excitation} = 488$ nm). Top: dark-field images; bottom: superposition of fluorescence and transillumination images.

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on the SiNW surface and forming SiNWs decorated with green-emitting QDs (green SiNWs). Notably, multicolor QD-decorated SiNWs could be achieved based on the quantum-size effect (red shift of emission wavelength of QDs with increasing dot size; E and F).[6] Indeed, as shown in Figure 1 b, green, yellow, and red SiNWs are obtained in our experiment by simply adjusting the reaction time and temperature (see Supporting Information for experimental details).

In comparison to the smooth surface of the free-standing SiNWs (Figure 2 a), distinct black dots (QDs) are observed on the surface of the red SiNWs with a maximum emission at about 620 nm (Figure 2 b,c). Furthermore, one single QD (indicated by the circle in Figure 2 c and enlarged in Figure 2 d to reveal the QDs) are spherical particles with an average size of about 4.5 nm, and are coupled to SiNW surface. Furthermore, the existence of well-resolved crystal lattices in the HRTEM image further confirms the highly crystalline structure of the QDs decorating the SiNWs. The diameter of the red SiNW is about 70 nm, which is smaller than that (100–200 nm) measured with a laser-scanning confocal microscope (LSCM; Figure 1 b). The reason for the decrease is that the amplification constant of LSCM is much smaller than that of TEM or SEM, leading to relatively unclear focus of the SiNW under LSCM.

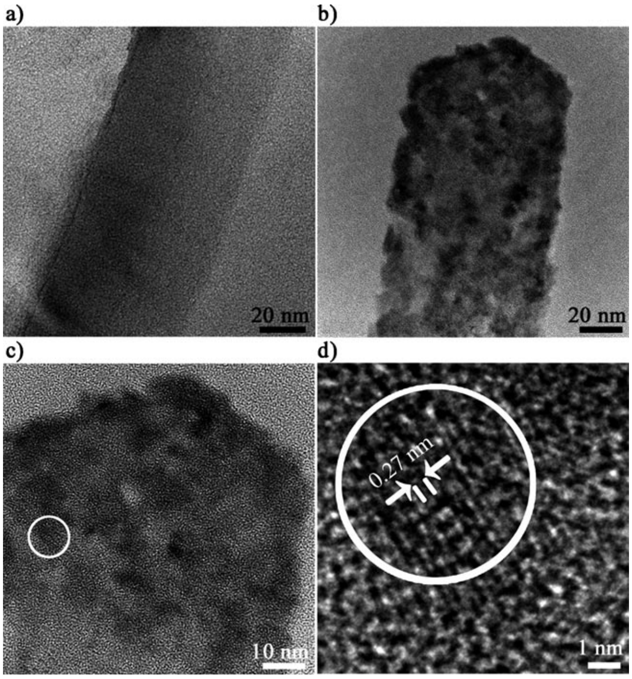

Figure 2. TEM/HRTEM overview images of a) a free-standing SiNW, and b–d) a red SiNW with a maximum emission at about 620 nm.

Figure 3 a and b shows the normalized absorption and photoluminescence (UV-PL) spectra of the resultant multicolor SiNWs with different maxima in the aqueous phase. In contrast to the precursor (that is, the initial reaction solution containing H-terminated SiNWs, MPA, and Cd^{2+} and Te^{2-} ions) with featureless absorption, the three colored SiNWs show a clear absorption peak of the first electronic transition at 430, 460, and 510 nm (Figure 2 a). Correspondingly, the SiNWs exhibit maximum PL peaks at 520, 555, and 620 nm (Figure 2 b), with strong blue, yellow, and red luminescence under UV irradiation (Figure 3 c). More importantly, the QDs-decorated SiNWs feature excellent aqueous dispersibility, which is due to the large amount of surface-covered MPA with negatively charged carboxylic groups.[5] Indeed, the resultant aqueous solution of SiNWs is highly transparent in ambient light, whereas the precursor solution exhibits severe turbidity (Figure 3 c). Of particular significance, the fluorescent SiNWs possess extremely robust photostability in addition to strong luminescence and excellent aqueous dispersibility.

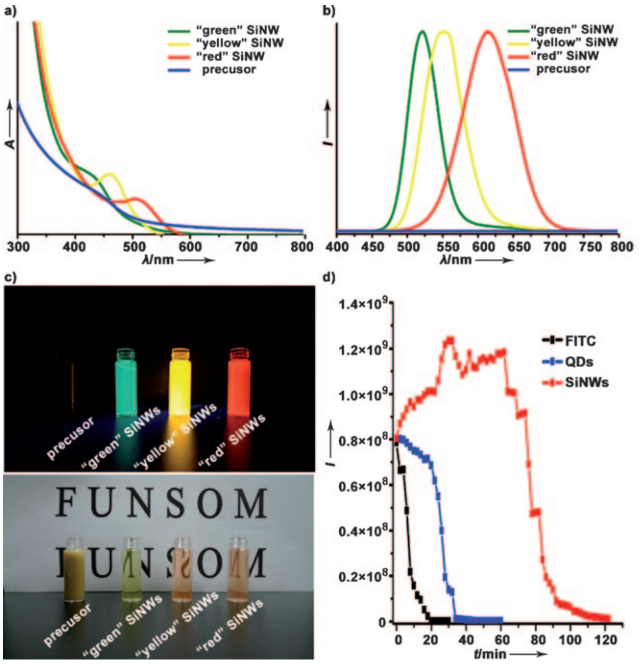

Figure 3. a) Absorption and b) photoluminescence (PL) spectra of the precursor, as-prepared green ($\lambda_{emission}=520$ nm), yellow ($\lambda_{emission}=555$ nm), and red ($\lambda_{emission}=620$ nm) SiNWs. c) Photographs of corresponding aqueous solutions under 365 nm irradiation (top) or ambient light (bottom). The samples were directly extracted from the original solution right before or after reaction without any post-treatment. d) Photostability comparison of FITC, CdTe QDs, and the red SiNWs.

We compared the photostability of FITC (fluorescein isothiocyanate) dye, free-standing CdTe QDs, and the as-prepared SiNWs in a parallel experiment. Figure 3 d shows that the fluorescence of FITC rapidly decreases below 50 % in about 5 mintes, and that of CdTe QDs, though more stable, becomes undetectable after about 30 minutes because unsaturated Te atoms on the CdTe QD surface are prone to oxidation under intense UV irradiation.[6c,d,7] In contrast, the fluorescence intensity of SiNWs is remarkably stable, preserving more than 50 % of the original intensity even after 80 minutes of high-power UV irradiation (Supporting Information, Figure S6). We attribute the remarkable PL stability to SiNWs serving as an effective substrate for binding to the in situ QDs.[3d] Such QDs coupled to SiNWs feature distinctively different electrochemical and optical properties to the free-standing QDs, leading to superior photostability.[8] Interestingly, the fluorescent intensity of the prepared SiNWs is obviously enhanced by 25–50 % in the initial 60 minutes of UV irradiation. This feature is consistent with a recent demonstration that SiNWs with metal nanocluster coatings is an effective nanoantenna to capture, confine, and enhance an optical signal.[9] Furthermore, the SiNWs may reduce the contribution of the non-irradiative channel for electron–hole recombination, thus enhancing the PL of the coupled QDs during the initial period of UV irradiation.[3d,5c,d,6d] As the irradiation proceeded beyond 80 minutes, the surfaces of QDs would be substantively oxidized, resulting in gradual fluorescence quenching.[6d,7]

Of particular importance is that such ultra-photostable, highly fluorescent, and water-dispersed SiNWs can be utilized for real-time and long-term immunofluorescent cellular imaging. The SiNWs can be readily conjugated with antibodies, as they contain a large number of surface carboxylic groups for conjugation with the amine groups of proteins.[10] Figure 4 shows Hela cells dually stained by the SiNW bioconjugates and a cell-nucleus dye Hoechst (a commercially available organic dye) with a high spatial resolution. Cellular microtubules and nuclei are differentially labeled by the red-colored SiNWs bioconjugates (Figure 4 a) and the blue-colored Hoechst, respectively (Figure 4 b). Notably, the photoluminescence of the SiNWs-labeled cellular microtubules is very bright and clearly spectrally resolved.

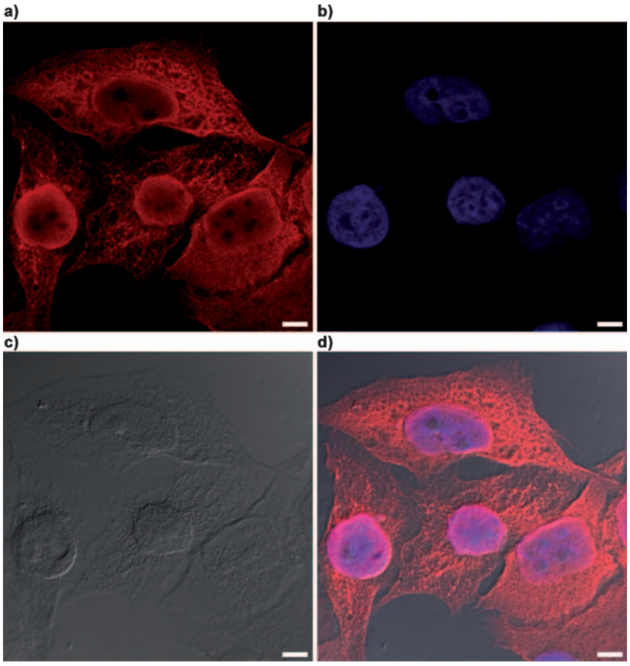

Figure 4. Photos of dual-color labeling cells captured by laser-scanning confocal microscopy. Hela cells are distinctively labeled by the red SiNWs/protein bioconjugates (red) and Hoechst (blue). a) Cellular microtubules are labeled by the red SiNWs/protein bioconjugates ($\lambda_{excitation}$ = 488 nm; window: 570–670 nm). b) Cellular nuclei are labeled by Hoechst (($\lambda_{excitation}$ = 405 nm; windows 415–485 nm). c) Bright-field image. d) Superposition of fluorescence and transillumination images. Scale bars: 5 μm.

We also compared the fluorescence evolution of organic dyes (FITC and Hoechst), II/VI QDs (CdTe QDs), and the SiNWs in long-term cellular imaging. Significantly, the fluorescence signals of SiNWs are remarkably stable against photobleaching compared to those of the fluorescent dyes and QDs. As shown in Figure 5 a, the green fluorescence signals of FITC rapidly diminish in 2 min owing to severe photobleaching. In comparison, the red fluorescence signals of the QDs are spatially resolved in an initial 10 min irradiation owing to a greater photostability than FITC, although the signals almost disappear at 20 min irradiation (Figure 5 b). Hoechst, as one of the most photostable organic dyes,[11] displays bright fluorescence in the first 10 min irradiation; nevertheless, the blue signals nearly vanish after 40 min irradiation (Figures 5 b–c). In sharp contrast, the SiNWs preserve a strikingly stable fluorescence against photobleaching throughout a long-time imaging period, in accordance with our above discussion on the excellent photostability of SiNWs. The red signals are persistently strong and even slightly brighter during 40 min observation (Figure 5 c), and is still distinctively observable up to 90 min after irradiation (Supporting Information, Figure S7).

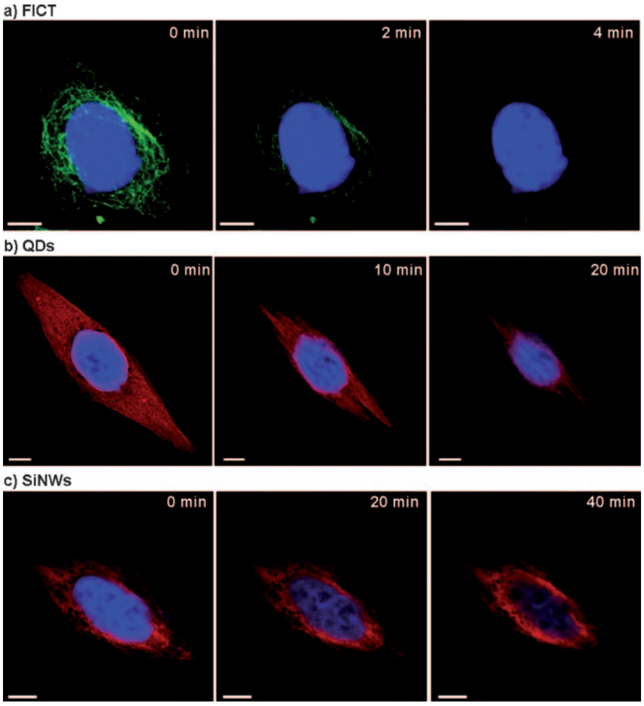

Figure 5. Stability comparison of fluorescence signals of Hela cells imaged by FITC, Hoechst, CdTe QDs, and SiNWs. Scale bars: 5 μm.

In summary, we present the first example of multicolor fluorescent SiNWs, which are facilely prepared by a one-pot microwave-assisted synthesis. Significantly, the SiNWs possess excellent aqueous dispersibility, strong photoluminescence, and ultrahigh photostability. Cellular experiments show that the as-prepared SiNWs are particularly suitable for long-term and real-time immunofluorescent cellular imaging. The unique advantages suggest the novel SiNWs may provide a new route for investigation of chemical/physical/optical properties of SiNWs and II/VI QDs, and also for various optoelectronic and biological applications.[4d,12]

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