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Quantum Dots

A General Route to Efficient Functionalization of Silicon Quantum Dots for High-Performance Fluorescent Probes

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Dedicated to Prof. Daoben Zhu on the occasion of his 70th birthday

***A** general technique for efficient surface modification of silicon nanocrystals is highly desirable for the development of silicon quantum dots (SiQDs) as fluorescent probes for biological applications. Herein, a facile microwave-assisted hydrosilylation process for the preparation of stable SiQDs in a single step is presented. FTIR spectroscopy indicates that molecules with various terminal functionalities, such as alcohol, alkyl groups, and carboxylic acid, are grafted successfully onto the surface of silicon nanocrystals. The dispersibility of such SiQDs is clearly dependent on the terminal functional groups of the grafted molecules. In addition, the as-prepared SiQDs show excellent cell compatibility, photoluminescence properties, and stability, and their use as long-term intracellular fluorescent probes is also demonstrated. It is envisaged that this facile and effective method for the stabilization and functionalization of SiQDs with tailored wetting and chemical properties will enable wide application of SiQDs in a number of areas.*

1. Introduction

Silicon quantum dots (SiQDs) have attracted great attention in various fields owing to their unique physical properties. In particular, the excellent biocompatibility and intrinsic low toxicity characteristics of SiQDs hold great promise for their application in biology and biomedicine.[1–8] Generally, the surfaces of freshly synthesized SiQDs are chemically active and can easily be subjected to oxidation and degradation.[9–15] In addition, the Si–O linkages have been shown to greatly affect the electronic charge distribution in the SiQDs.[16] It is therefore essential to further modify the surface of SiQDs to realize their functionalities. Surface modification of SiQDs can not only increase their stability and dispersibility in certain solvents, but also provide opportunities for their further functionalization and enhance their physical properties in some cases. For biomedical applications,[17–19] the SiQDs are required to have a fast radiative recombination rate, a substantial photoluminescence (PL) quantum yield in the visible region, and be water dispersible and hydrophilic to prevent aggregation and precipitation in biological microenvironments.[20–22]

A number of attempts have been made to modify the surfaces of SiQDs using the Si–C bond due to its thermodynamic and kinetic stability arising from its high bond strength and low polarity. Current methods for SiQD surface modification via the Si–C bond involve hydrosilylation with the use of a compound containing unsaturated C=C groups under UV light irradiation, thermal inducement, or a platinum-catalyzed initiation.[23–28] However, each approach has its own limitations. For example, photochemical hydrosilylation

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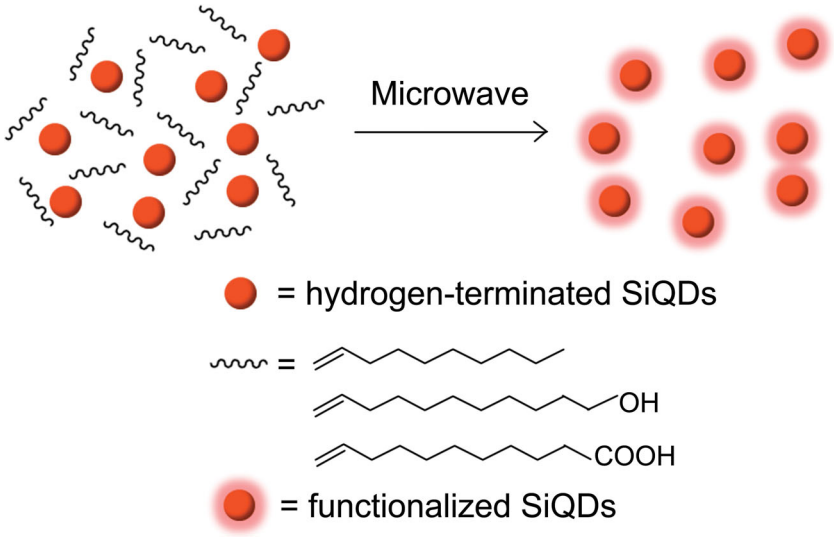

Scheme 1. Overall procedure for the preparation of SiQDs functionalized with decene, undecylenyl alcohol, and undecenoic acid.

requires the use of UV light for hours, which would cause adverse effects on the optical properties of SiQDs.[29] Similarly, thermally induced hydrosilylation on the SiQD surface could occur in the absence of initiator at higher temperatures; this reaction took several hours to give a high surface coverage. Pt complexes and colloids are effective catalysts for the hydrosilylation of alkenes with the hydrogen-terminated SiQD surface; however, Pt complexes are toxic and expensive, and they will inevitably adsorb on to the QDs. A facile method facilitating efficient modification of SiQDs while preserving/enhancing their intrinsic properties is very desirable.

Herein, we present an approach for producing stable silicon nanoparticles with various surface functionalities through microwave-assisted hydrosilylation of hydride-terminated silicon nanoparticles in the presence of various reactive compounds. Reagents were selected from compounds with bifunctional groups. One functional group may be the C=C that was involved in the hydrosilylation process and the other group could bear any other functionality to enable widespread applications of SiQDs. As illustrated in **Scheme 1**, stabilized SiQDs with various surface functionalities were produced through a simple microwave process using a mixture of hydrogen-terminated SiQDs and alkene-bearing functional molecules in a solvent. A remarkable advantage of using microwave-assisted modification of SiQD surfaces is that the rate of hydrosilylation reaction is fast and the achieved surface coverage is high, and as a result, the stability of the SiQDs can be significantly increased. That the whole procedure could be completed within 15 min indicated that this approach was more efficient than any of the existing methods. The carboxylic acid-terminated water-dispersible SiQDs were further used as effective biological labels for cell fluorescence imaging due to their low toxicity.[30]

2. Results and Discussion

Essentially, all the hydrogen-terminated silicon nanoparticles can be functionalized through microwave-assisted chemical reaction. Perhaps the most widely studied silicon-based nanostructure is hydrogen-terminated porous silicon. The point of using porous silicon as a starting material is that it ensures the purity of the silicon core within the nanocrystal.

Figure 1a is a transmission electron microscopy (TEM) image of suspended silicon nanoparticles produced by ultrasonicating porous silicon in ethanol. The nanostructures in the range of 10 to 50 nm are not individual nanocrystals, but are

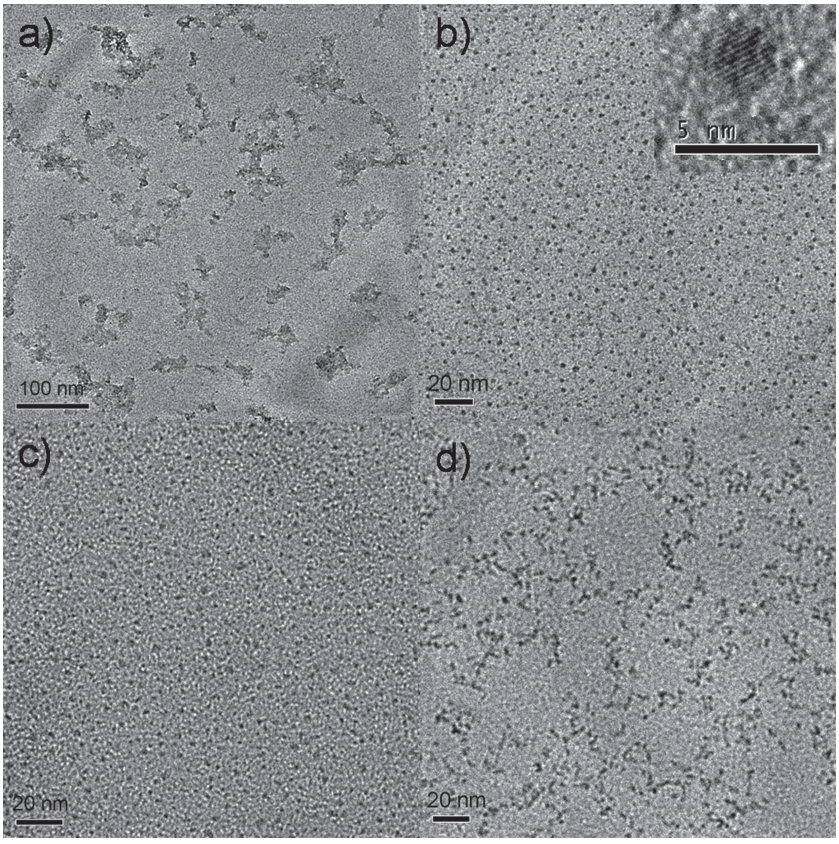

Figure 1. TEM images of a) freshly prepared hydrogen-terminated silicon nanoparticles, b) undecylenyl alcohol-functionalized SiQDs (inset: high-resolution TEM image of an individual silicon nanocrystal), c) undecenoic acid-functionalized SiQDs, and d) decene-functionalized SiQDs.

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silicon nanoparticle domains trapped in larger pieces of the porous silicon structure. The PL property of the silicon nanofragments is the same as that of the original porous silicon film, and the nanofragments can be used for the further functionalization step. Figure 1b displays the TEM image of the undecylenyl alcohol-functionalized SiQDs and the inset shows a high-resolution TEM image of an individual silicon nanocrystal. Due to the thermal effect of the microwave irradiation and efficient surface modification, silicon nanofragments separate into individual silicon nanoparticles with an average diameter of 4 ± 0.5 nm. The TEM image (Figure 1c) of undecenoic acid-functionalized SiQDs is identical to that of the undecylenyl alcohol-functionalized ones, and the good water dispersibility of both types of SiQDs indicated the effective grafting of molecules on to the silicon cores. Note that the TEM image of the decene-functionalized SiQDs shown in Figure 1d displays slightly clustered SiQDs with an average size of 4 ± 1 nm.

It should be noted that if the functionalization of SiQDs with 1-decene was carried out in the same way as for the preparation of undecylenyl alcohol- and undecenoic acid-functionalized SiQDs, it led to a less efficient chemical grafting of alkyl chains on the silicon nanocrystals. This behavior may be related to the very weak polarity of 1-decene, as the ability for microwave energy absorption depends heavily on the polarity of the reagents. When functional alkenes bearing a polar group such as organic acid or alcohol were treated with microwaves, the rise in temperature was very clear due to the absorption of microwave energy by polar molecules. This allowed the formation of "hot" centers around silicon nanocrystals, which enabled hydrosilylation to take place. For the grafting of 1-decene molecules, although silicon nanocrystals can absorb microwave energy efficiently, it was transferred to the medium rapidly and the local temperature around the SiQDs did not always reach that required for the hydrosilylation reaction. Since the porous silicon substrate absorbs microwave irradiation more efficiently than silicon nanoparticles, a slightly modified procedure was employed for the grafting of 1-decene: the freshly prepared porous silicon was immersed in 1-decene and subjected to microwave irradiation, followed by ultrasonication in hexane to afford the luminescent alkyl-modified SiQDs.

The SiQDs prepared as above were purified and dried under vacuum, and KBr pellets of each silicon nanoparticle powder were prepared for Fourier transform infrared (FTIR) analysis. **Figure 2**a shows the FTIR spectrum of decene-functionalized silicon nanoparticles. The spectrum reveals characteristic C–H stretching signals, with the symmetric CH_2, antisymmetric CH_2, and asymmetric CH_3 stretching vibrations at 2856, 2926, and 2960 cm^{-1}, respectively. Two observed peaks at 1461 and 1261 cm^{-1} are attributed to the vibrational scissoring and symmetric bending of $Si–CH_2$, respectively. The two peaks can be seen in all three spectra, thus confirming that the organic group is indeed bound covalently to the silicon nanoparticle surface via a silicon–carbon bond. No evidence of C=C stretching (1615 and 1645 cm^{-1}) is present after purification, which indicates the full removal of capping molecules. In the spectrum of hydroxyl-terminated silicon nanoparticles (see Figure 2b), the peak corresponding to the typical O–H stretching of the alcohol group can be observed at 3350 cm^{-1}. Figure 2c displays the FTIR spectrum

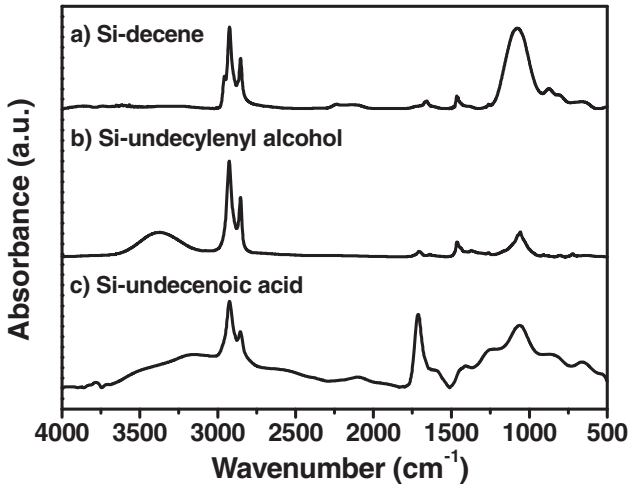

Figure 2. FTIR spectra of a) decene-, b) undecylenyl alcohol-, and c) undecenoic acid-functionalized SiQDs.

of undecenoic acid-functionalized silicon nanoparticles. The characteristic carboxylic acid C=O feature arising from surface-tethered organic acids appears at 1715 cm^{-1}. The peak observed at 3150 cm^{-1} is attributed to the stretch vibration of the O–H bond of a carboxylic acid group. The FTIR results of functionalized SiQDs also indicated that these functional groups have very good stability under microwave irradiation.

The optical properties of decene-, undecylenyl alcohol-, and undecenoic acid-functionalized SiQDs were investigated using UV–visible and PL spectroscopy (**Figure 3**a). The UV–visible spectra of undecylenyl alcohol- and undecenoic acid-functionalized SiQDs, which were prepared from hydrogen-terminated SiQDs, give a similar broad absorption band with a shoulder at 320 nm characteristic of silicon nanoparticles. Correspondingly, the fluorescence emission spectra of hydroxyl-terminated and carboxylic acid-terminated SiQDs in water reveal a red luminescence centered at 670 and 680 nm, respectively. The apparent blueshifted PL band is from decene-modified SiQDs. When the SiQDs were prepared from decene-functionalized porous silicon, a blue emission around 400 nm was observed for the initially red luminescing porous silicon at 645 nm. This blue emission is believed to arise from the oxidation-induced defects at the surface of porous silicon, which are attributed to the different procedure for the microwave-assisted functionalization of the two types of SiQDs. It was known that upon microwave irradiation, the temperature of porous silicon substrate increased greatly. As a result, hydrogen-terminated porous silicon may be easily oxidized by the trace amount of oxygen in water that may have been trapped inside the micro/nanostructures. Another possibility was that during the sonication process, to release the SiQDs into solvent, some Si–Si bonds were broken and formed new active sites which were very sensitive to oxygen or water molecules.

To verify this hypothesis, we prepared undecylenyl alcohol-functionalized porous silicon in the same way as the decene-grafted SiQDs. The PL result of these hydroxy-terminated SiQDs, unsurprisingly, shows an identical PL spectrum to that of decene-functionalized SiQDs. In addition, the intensity of the blue emission from oxidized SiQDs

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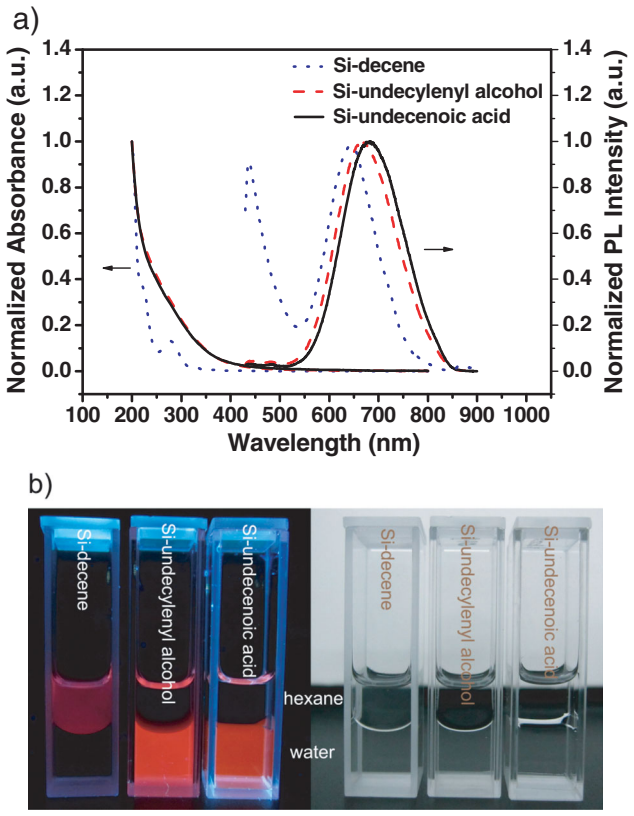

Figure 3. a) Absorption and PL spectra of decene-, undecylenyl alcohol-, and undecenoic acid-functionalized SiQDs. PL is measured using ultraviolet excitation (λ = 365 nm). b) Photographs of SiQDs functionalized with 1-decene (left) dispersed in hexane, and with undecylenyl alcohol (middle) and undecenoic acid (right) dispersed in water under 365 nm irradiation or ambient light.

is decreased after several days whereas the red emission from the undecylenyl alcohol-coated SiQDs was essentially unchanged (Supporting Information, Figure S1). Figure 3b shows photographs of SiQDs functionalized with 1-decene, undecylenyl alcohol, and undecylenic acid dispersed in a mixed solvent of hexane and water, under UV illumination (left) and bright field (right). It can be seen that after the completion of phase separation of the solvents, both the upper layer (hexane) and lower layer (water) are transparent to the naked eye for all three differently functionalized SiQDs due to their low absorbance coefficient in the visible region and low concentration, whereas under UV light excitation the QDs emitted bright red light. Clearly, decene-functionalized SiQDs were only dispersed in the hexane layer and the others were dispersed in the water layer. The PL characteristics of the materials were stable, at least over a few months, and they exhibited very good stability in organic solvent or water.

To demonstrate the intrinsic PL properties of water-dispersible SiQDs in biological systems, we incubated undecenoic acid-functionalized SiQDs with human cervical adenocarcinoma cell line (HeLa) for in vitro cellular imaging. As the confocal microscopy images shown in **Figure 4** indicate, strong fluorescence intensity was recorded after 24 h of incubation, which indicates that a large number of SiQDs were internalized. These nanoparticles displayed a cytoplasmic distribution in the cells and did not show a significant accumulation in the nucleus. The internalized SiQDs kept significant luminescence and did not quench after exposure to a laser with an excitation wavelength of 405 nm for 1 h. Thus, the water-dispersible SiQDs can be employed as an excellent long-term intracellular fluorescent probe.

Cell viability assay was performed for cytotoxicity evaluation. **Figure 5** shows the in vitro results for the water-dispersible SiQDs including undecylenyl alcohol- and undecenoic acid-functionalized SiQDs. It is clear that the SiQDs show no toxicity to HeLa cells in vitro within the tested concentration range at 24 and 48 h post-treatment.

3. Conclusion

In summary, a general and efficient method for the production of surface-functionalized SiQDs has been developed. Functionalization of hydrogen-terminated silicon nanoparticles under microwave irradiation has been performed to produce nanoparticles with various surface functionalities such as alkyl, alcohol, and carboxylic acid. These SiQDs exhibit excellent luminescence properties and show very good stability in organic or aqueous media. In comparison with previous methods utilized for the surface modification of SiQDs, the microwave-assisted hydrosilylation process contributes several advantages including simpler workup procedure, shorter reaction time, milder conditions, and environmental friendliness. Establishing this efficient microwave-assisted functionalization method as being flexible toward various

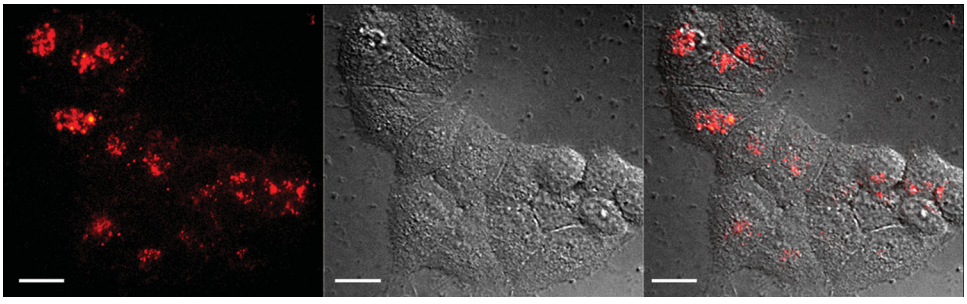

Figure 4. Confocal microscopy images of undecenoic acid-functionalized SiQDs in HeLa cells. Images are representative of more than three independent experiments. From left to right: luminescence image, bright-field image, and the overlay of the two. Scale bar = 10 µm.

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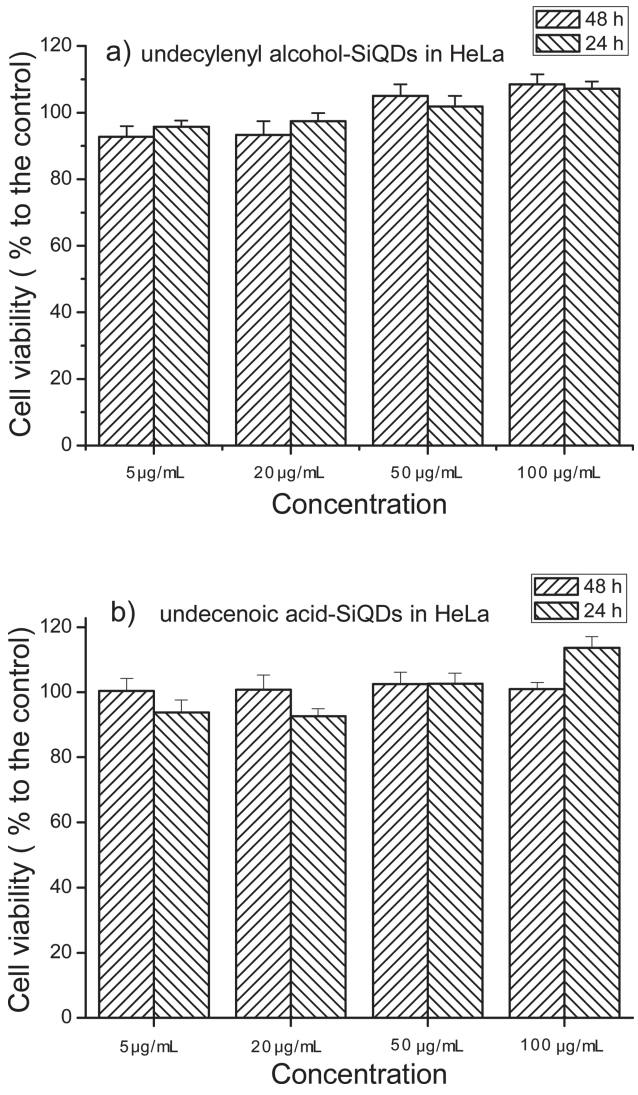

Figure 5. Cytotoxicity of a) undecylenyl alcohol- and b) undecenoic acid-functionalized SiQDs toward HeLa cells.

functionalities serves to increase the potential applications of SiQDs.

4. Experimental Section

Materials: 1-Decene (96%), omega-undecylenyl alcohol (99%), and 10-undecenoic acid (99%) were purchased from Alfa Aesar. All chemicals were used as received without additional purification.

Production of Hydrogen-Terminated Silicon Nanoparticles: The single side polished, (100) oriented, p-type Si wafers (B doped, 7–9 Ω cm resistivity) were boiled in 7:3 (v/v) concentrated H_2SO_4/30% H_2O_2 for 30 min, and then rinsed copiously with Milli-Q water. The electrolyte of the electrochemical etching process was an ethanolic HF solution (1:1 (v/v) 40% HF/EtOH). A Pt ring and Si wafer were placed into the electrolyte and connected to a dc power supply by wires. SiQDs were obtained in an etching process with a current density of 130 mA cm^{-2} for 10 min, and were well dispersed in ethanol solution (20 mL) by ultrasonic treatment. The precipitates after ultrasonication were removed using a 0.22 μm PVDF syringe filter (Millipore). Afterwards, the ethanol solution of purified SiQDs was utilized for further functionalization.

Microwave-Assisted Functionalization of SiQDs: Microwave irradiation was performed in an LWMC-205 reactor with an adjustable power of 0–750 W and operating at a frequency of 2450 MHz. For the synthesis of decene-functionalized SiQDs, the freshly prepared porous silicon was immersed in a pure 1-decene solution (20 mL) for microwave irradiation, followed by ultrasonication in ethanol. After being isolated by filtration and dried under vacuum, the obtained SiQDs were dispersed in hexane. For undecylenyl alcohol- and undecenoic acid-functionalized SiQDs, the reactants of the microwave-assisted chemical reaction were dispersions of hydrogen-terminated Si nanoparticles in ethanol (20 mL) containing the corresponding grafting molecules. The resulting SiQDs were purified by dialysis against alcohol (molecular weight cutoff (MWCO) 7000, SERVA, Membra-Cel dialysis tubing, diameter 22 mm) to remove residuals of unreacted grafting molecules.

Cell Imaging: HeLa cells were plated onto 30-mm cell culture coverslips and incubated with undecenoic acid-functionalized SiQDs (20 μg mL^{-1}) for 24 h. The attached nanoparticles were washed three times with phosphate-buffered saline (PBS, pH 7.0) and cells were monitored with an UltraVIEW VOX confocal system (PerkinElmer Co.) using a 60 × 1.4 numerical aperture (NA) oil-immersion objective lens excited at 405 nm.

Cell Viability Assays: Cell viability was evaluated with CCK-8 assay kits (Dojindo Laboratories, Japan). About 5×10^3 cells were plated onto 96-well plates (Corning, USA). After incubation with different concentrations of SiQDs for specific time points (24, 48 h) at 37 °C, CCK-8 reagents were added according to the manufacturer's protocol and incubation was carried out for 2 h. The absorbance was measured at 450 nm with a subtraction for reference at 600 nm by an Infinite M200 microplate reader (Tecan, Durham, USA). The mean absorbance of nonexposed cells was taken as the reference value (100% cellular viability). Data are representative of three independent experiments with at least three wells per treatment.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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