

Water-Soluble Luminescent Silicon Nanocrystals by Plasma-Induced Acrylic Acid Grafting and PEGylation

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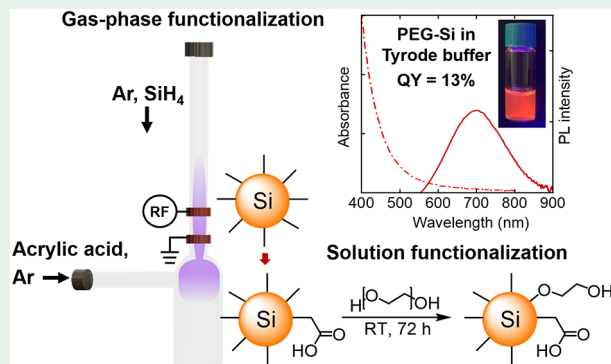
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Supporting Information

ABSTRACT: Luminescent silicon nanocrystals are promising nano-materials for biomedical applications due to their unique optical properties and biocompatibility. Here, we demonstrate a two-step surface modification approach coupling gas-phase and liquid-phase methods to synthesize PEGylated acrylic acid grafted silicon nanocrystals with near-infrared emission in water and biological media. First, acrylic acid grafted silicon nanocrystals are synthesized by an all-gas-phase approach on a millisecond time scale, omitting high temperature and postpurification processes. Subsequently, room-temperature PEGylation is carried out with these acrylic acid grafted silicon nanocrystals, yielding stable colloidal dispersions in both water and high ionic strength Tyrode's buffer with 20–30 nm hydrodynamic diameters. The PEGylated silicon nanocrystals exhibit photoluminescence in the 650–900 nm near-IR window with quantum yields of ~30% and ~13% in deionized water and Tyrode's buffer, respectively, after a 7-day oxidation in water. The surface-functionalized Si NCs exhibit relatively small toxicity to MDA-MB-231 cells at concentrations relevant to bioimaging applications.

KEYWORDS: silicon nanocrystals, plasmas, photoluminescence, quantum dots, colloidal stability, bioimaging



INTRODUCTION

Inorganic nanocrystals have garnered increasing interest in biomedical applications, such as drug and gene delivery,¹ biosensing,² and bioimaging.^{3,4} Specifically, silicon nanocrystals (Si NCs) are desirable for biomedical applications because of their low cytotoxicity,^{5,6} biocompatibility,⁷ biodegradability,⁸ and size-tunable optical properties.^{9,10} Similar to silica or porous silicon, Si NCs are hydrolytically unstable and dissolve over time into water-soluble silicic acid ($\text{Si}(\text{OH})_4$),¹¹ which naturally occurs in human tissues and can be excreted through renal clearance.^{12,13} In addition, their size-tunable emission in the biologically relevant red-to-near-infrared window and high photostability makes them a potential alternative for heavy metal based quantum dots.¹⁴

Various strategies have been applied to synthesize luminescent water dispersible Si NCs, which can be mainly categorized into steric stabilization and electrostatic stabilization. The use of polymers such as polyethylene glycol derivatives typically provides steric stabilization.^{14–16} Electrostatic stabilization approaches typically rely on ionic ligands including olefinic ligands with terminal carboxylic acid^{17–19} or amine functional groups.^{20–22} The colloidal stability of such surface-functionalized Si NCs in water or high ionic strength buffer solutions greatly depends on the type of stabilization strategy. Compared to steric stabilization, stability achieved by

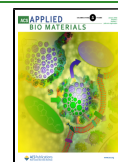
electrostatic repulsion is more strongly affected by pH or ionic strength.^{23–25}

In this work, we explored a two-step synthetic procedure coupling steric and electrostatic repulsions to achieve colloidal stability of Si NCs in both water and high ionic strength buffer that is similar to biological media. First, we synthesized acrylic acid grafted Si NCs using an all-gas-phase approach on a millisecond time scale without any postsynthetic processing. Plasma-assisted surface functionalization with alkene ligands to produce luminescent Si NCs has been demonstrated earlier, and a similar approach was employed to synthesize acrylic acid grafted Si NCs by adjusting the plasma power and gas flow rates.^{26,27} Acrylic acid or poly(acrylic acid) coating of Si NCs has been extensively studied in the solution phase to generate a hydrophilic surface and to reduce nanoparticle agglomeration in water or biological media.^{17–19,28,29} Acrylic acid ligands with a terminal carboxylic acid group stabilize particles through a combination of steric stabilization and electrostatic repulsion, thus the colloidal stability can be affected by the presence of

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electrolytes and pH of the medium.^{23,24,29–31} Hence, even though acrylic acid grafting improves the nanocrystal dispersibility in water, the functionalized nanoparticles are typically slightly agglomerated with hydrodynamic diameters around 100 nm.

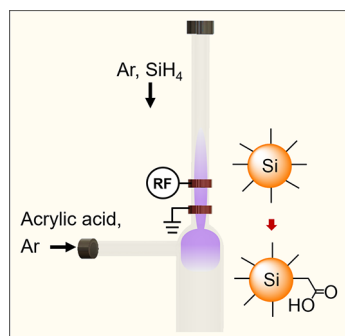
In the second step, the acrylic acid grafted Si NCs were reacted with polyethylene glycol (PEG) to achieve steric stabilization. PEG, being safe and nontoxic with nearly neutral surface charges, is one of the most popular and most effective molecules for coating the surface of nanocrystals.^{32–35} Surface PEGylation of Si NCs has been achieved by utilizing covalent Si-C bonds,^{36,37} Si-O linkage,³⁸ chloride-terminated silicon surfaces,³⁹ and boron surface sites.⁴⁰ As the acrylic acid grafting enables an improved dispersibility in polar solvents that allows for large initial surface areas available for reactions, we can employ the reaction with PEG-OH to form Si-O-R linkages at room temperature.⁴¹

Herein, we report the synthesis of luminescent PEGylated acrylic acid grafted Si NCs combining gas-phase and colloidal approaches under room temperature conditions. The synthesis route avoids high temperatures that have been typically used and requires a minimal amount of solvent. The surface functionalized nanocrystals exhibit excellent dispersibility in both water and a high ionic strength buffer media maintaining colloidal stability for months. Furthermore, after oxidation in deionized (DI) water, they exhibit photoluminescence quantum yield (PLQY) up to ~30% in DI water and ~13% in Tyrode's buffer in NIR emission wavelengths, which is suitable for *in vivo* imaging.

RESULTS AND DISCUSSION

Acrylic acid grafted Si NCs (AA-Si NCs) were synthesized using an all-gas-phase setup as illustrated in Scheme 1. Si NCs

Scheme 1. Schematic of the Synthesis of Acrylic Acid Grafted Si NCs



were synthesized in a higher density upstream plasma region followed by the acrylic acid ligand grafting, which occurs at the downstream “afterglow” region or the lower density plasma region. The plasma induced synthesis and surface functionalization occur on a time scale of 10 ms, which is significantly faster than a typical colloidal synthesis of acrylic acid grafted Si NCs. The AA-Si NCs were transferred into water and Tyrode's buffer through ultrasonication.

As-synthesized AA-Si NCs have crystalline cores of ~3 nm determined by Scherrer analysis (Figure S1). We performed Fourier-transform infrared (FT-IR) spectroscopy and X-ray photoelectron spectroscopy (XPS) measurements to characterize the surface composition of the AA-Si NCs. Figure 1a shows

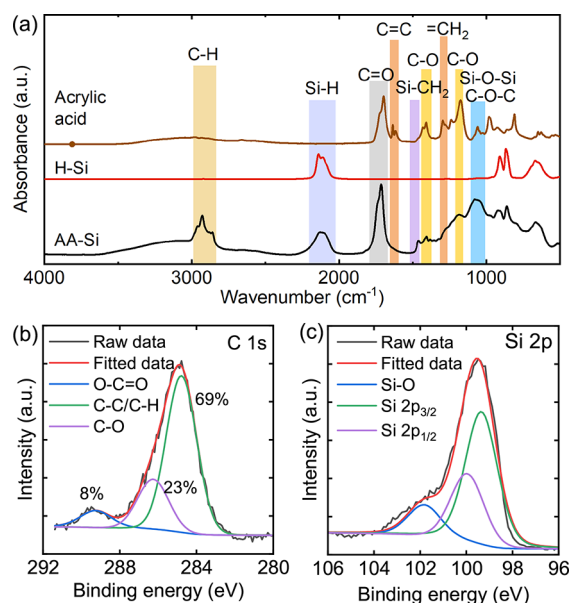


Figure 1. Characterization of acrylic acid grafted Si NCs. (a) FTIR spectra of acrylic acid, H-Si NCs, and AA-Si NCs. XPS high-resolution spectra of (b) C 1s peak and (c) Si 2p peaks. The peak corresponding to adventitious carbon is shifted to 284.8 eV as a reference.

the FT-IR spectra of acrylic acid, hydrogen-terminated Si NCs (H-Si NCs), and AA-Si NCs. The H-Si NCs were produced under the same synthesis conditions but without acrylic acid injection to the flow stream. The FT-IR spectrum of AA-Si NCs exhibits features at 2200–2100 cm^{-1} , ~1100 cm^{-1} , 1700–1800 cm^{-1} , and 2800–3000 cm^{-1} , which were assigned to Si-H stretching, Si-O-Si asymmetric stretching, C=O stretching, and C-H stretching modes, respectively. The disappearance of the C=C stretching peak at 1640 cm^{-1} and emergence of Si-CH₂ peaks at 1460 cm^{-1} suggests that the hydrosilylation reaction proceeds as expected, replacing C=C and Si-H bonds with Si-C bonds.

As shown in Figure 1b,c, XPS data further confirm the effective surface functionalization of AA-Si NCs. The survey XPS spectrum (Figure S2) confirms the presence of Si, C, and O with atomic concentrations of 33%, 46%, and 21% respectively. The C 1s high-resolution spectrum exhibits a peak at 288.8 eV, which is consistent with the COOH/COOR functionalities, indicating the retention of carbonyl groups in acrylic acid grafted Si NCs. The COOH/COOR concentration is around 8%, which is typical among the other studies of acrylic acid plasma coatings.^{42,43} In accordance with the Si 2p high-resolution spectrum, the peak at 99.4 eV corresponds to the Si(0) component and two shoulders located at 100.1 and 101.9 eV indicate the oxidized Si species.

The hydrophilic nature of AA-Si NCs enables the dispersion of functionalized particles in polar organic solvents and water.^{17,18} The improved dispersibility of AA-Si NCs can be attributed to a combination of steric stabilization and electrostatic stabilization induced by negative surface charges.^{28,29} Similarly, plasma-synthesized AA-Si NCs spontaneously form a clear and stable solution in methanol and are readily dispersible in both water and Tyrode's buffer. Hydrodynamic diameters of AA-Si NCs in both water and Tyrode's buffer were evaluated using dynamic light scattering (DLS) measurements. AA-Si NCs have hydrodynamic

diameters of 67 ± 20 nm in water and 48 ± 12 nm in Tyrode's buffer (Figure S3). However, AA-Si NCs in Tyrode's buffer degrade completely within 2 h. Furthermore, they do not exhibit photoluminescence when introduced to either water or Tyrode's buffer.

Having demonstrated that AA-Si NCs exhibit improved dispersibility in water and Tyrode's buffer but with low stability and negligible photoluminescence (PL), we carried out a room temperature colloidal PEGylation reaction to achieve enhanced photoluminescence along with long-term stable dispersions. Briefly, AA-Si NCs were dispersed in a mixture of methanol and PEG-OH (molecular weight = 400), and methanol was subsequently evaporated. Then, AA-Si NCs and PEG-400 were allowed to react at room temperature for 72 h. The PEGylation reaction with a partially oxidized Si surface is expected to occur via the covalent reaction of the $-\text{OH}$ group with the Si-H^{41} or Si-O-Si^{44} on the Si NC surface.

We carried out FT-IR analysis of PEGylated AA-Si NCs to gain further information about the surface chemical conditions. As shown in Figure 2b, the FT-IR spectrum of the PEGylated

PEGylated AA-Si NCs in both water and Tyrode's buffer were evaluated using DLS. The hydrodynamic diameter in water and Tyrode's buffer were both measured to be 24 ± 6 nm (Figure 3a). The hydrodynamic diameters increased from 24 ± 6 nm to 36 ± 7 nm after 7 days of dispersion in water, indicating minor agglomeration during this period.

The hydrodynamic diameters were confirmed by transmission electron microscopy (TEM) measurements (Figure 3b,c). For TEM characterization, PEGylated AA-Si NCs were dispersed in water or Tyrode's buffer at 0.5 mg/mL concentration and drop-cast onto TEM grids. TEM images from both water and Tyrode's buffer dispersed PEGylated AA-Si NCs show small agglomerates within the size range of 20–30 nm. These results imply that PEGylated AA-Si NCs are not individually passivated but rather small PEG encapsulated nanocrystals.

To study the effect of having an acrylic acid grafted surface prior to PEGylation, we carried out a control experiment with pristine H-terminated Si NCs. The reaction was carried out under identical experimental conditions with hydrophobic Si NCs. We observed instant precipitation of PEGylated H-terminated Si NCs when introduced into water or Tyrode's buffer without forming a stable dispersion. This result suggests that the acrylic acid surface is crucial for effective surface functionalization with PEG-400 ligands, yielding stable dispersions in water and Tyrode's buffer.

On the basis of the above observations, we postulate that the hydrophilic nature of the acrylic acid grafted surface enables better dispersibility in methanol, allowing large initial surface areas available for PEGylation reaction. To test this hypothesis, AA-Si NCs with varying surface ligand coverages were synthesized by adjusting the distance between the powered electrode and the acrylic acid injection point. This distance was fixed at 3–4 cm for our typical experiments. Varying the distance between the electrode and the injection point affects the electron density where the ligands meet the nanoparticles, which ultimately influences the ligand grafting reactivity. As indicated by the decrease of the $\text{C}=\text{O}$ absorption at $1700\text{--}1800\text{ cm}^{-1}$ relative to the SiH_x absorption at $\sim 2100\text{ cm}^{-1}$ seen in Figure 4a, the surface ligand coverage decreases as the distance from the electrode to the injection point increases. As shown in Figure 4b, there is also a clear trend between the electrode-to-injection distance and solvodynamic diameters in methanol. We observed that the solvodynamic diameters of AA-Si NCs increased when the electrode-to-injection distance increased, which suggests a varying degree of acrylic acid surface functionalization. Furthermore, these results directly correlate with the hydrodynamic diameters in water after

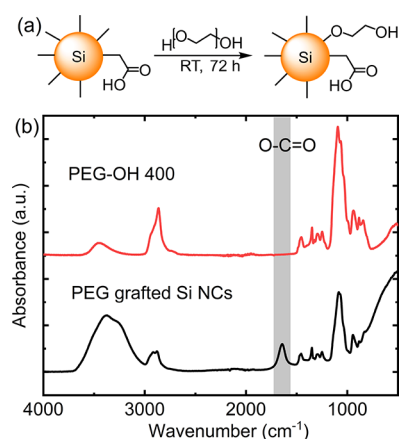


Figure 2. (a) Schematic of the PEGylation reaction and (b) FT-IR spectra of PEG-OH 400 and PEGylated AA-Si NCs.

AA-Si NCs is mainly dominated by the PEG ligand features. While the characteristic Si-H peaks diminished in the spectrum, the peak at 1700 cm^{-1} confirms the existence of $\text{C}=\text{O}$ species. This observation confirms the presence of PEG-400-grafted AA-Si NCs.

Purified PEGylated AA-Si NCs form a clear dispersion when introduced into water or Tyrode's buffer at 0.5 mg/mL, and these dispersions remain colloidally stable for months without any visible precipitation. Hydrodynamic diameters of

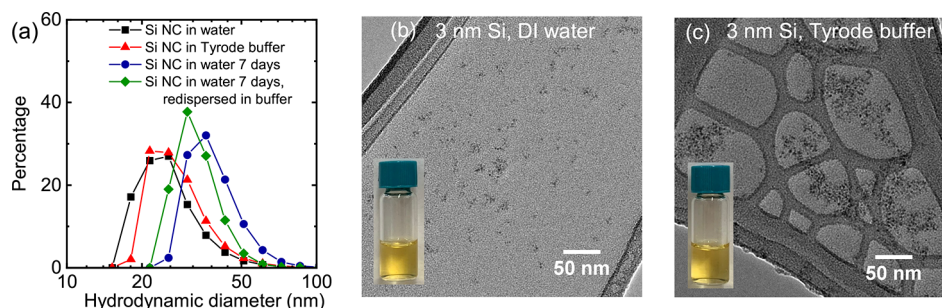


Figure 3. (a) DLS of PEG-Si NCs dispersed in water, Tyrode's buffer, water after 7 days, and water for 7 days and then redispersed in Tyrode's buffer, each at 0.5 mg/mL. TEM of PEG grafted Si NCs dispersed in water (b) and buffer (c).

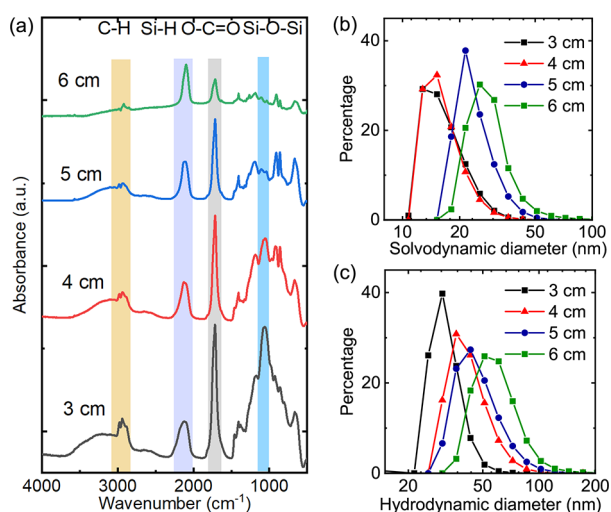


Figure 4. Effect of varying the electrode-to-ligand injection distances at 3, 4, 5, and 6 cm. (a) FTIR spectra of as-synthesized, acrylic acid grafted Si NCs. (b) Solvodynamic diameters of samples dispersed in methanol at 1 mg/mL. (c) Hydrodynamic diameters of these samples after PEGylation and dispersion in water, each at 0.5 mg/mL.

PEGylation reaction (Figure 4c). As the solvodynamic diameters in methanol range from 16 ± 4 nm for the 3 cm sample to 27 ± 6 nm for the 6 cm sample, the hydrodynamic diameters in water after PEG functionalization vary from 24 ± 6 nm to 56 ± 14 nm. These results confirm that acrylic acid grafting enables a better dispersion in methanol, which will directly affect the PEGylation reaction and hydrodynamic diameters in water.

Near-infrared (NIR) optical imaging offers improved contrast and depth of tissue penetration by reducing photon scattering, lowering tissue absorption, and minimizing

autofluorescence in the NIR window.⁴⁵ We examined the optical properties of PEGylated AA-Si NCs for their potential viability as bioimaging molecules. While PEGylated Si NCs in water exhibit PL centered at 775 nm with a photoluminescence quantum yield (PLQY) of 6% (Figure 5a), they exhibit PL at 775 nm with a PLQY of ~1% in Tyrode's buffer. Figure 5b–e shows the evolution of PL, PLQY, and peak wavelength of PEGylated AA-Si NCs in water (Figure 5b–d) and Tyrode's buffer (Figure 5e). As shown in Figure 5d,e, the PL wavelength maxima blueshift and the PLQY increases over time in both water and Tyrode's buffer. The continued PLQY increase coupled with PL maxima blue shift indicate surface oxidation and effective passivation of the Si NCs with an oxide layer. However, PLQY of PEGylated AA-Si NCs are significantly different in water and Tyrode's buffer as they exhibit a 30% PLQY in water after 7 days and only 6% PLQY in the Tyrode's buffer. In addition, discoloration was observed in PEGylated AA-Si NC dispersion in the Tyrode's buffer, indicating a significant degradation of the Si NCs due to particle oxidation or dissolution. The degraded PLQY is presumed to originate from charge transfer processes to the ions present in the buffer.⁴⁶ Electrolytes are also known to accelerate the dissolution of silicon oxide^{47,48} and thus remove the passivating oxide shell and create defects. As can be observed in Figure 5d,e, the PL maxima blueshift occurs much faster in Tyrode's buffer than in water, indicating more rapid oxidation in the buffer. The PL spectra of PEGylated AA-Si NCs in water exhibit Gaussian profiles with the fwhm's remaining relatively constant at ~175 nm (Figure 5f).

Aged PEGylated AA-Si NCs in water exhibit higher PLQY that can be ascribed to the formation of a protective oxide shell that develops an enhanced stability against PL quenching and oxidation. In order to explore the colloidal stability and PL behavior of aged and oxidized PEGylated AA-Si NCs in Tyrode's buffer, we took a dried sample of PEGylated AA-Si

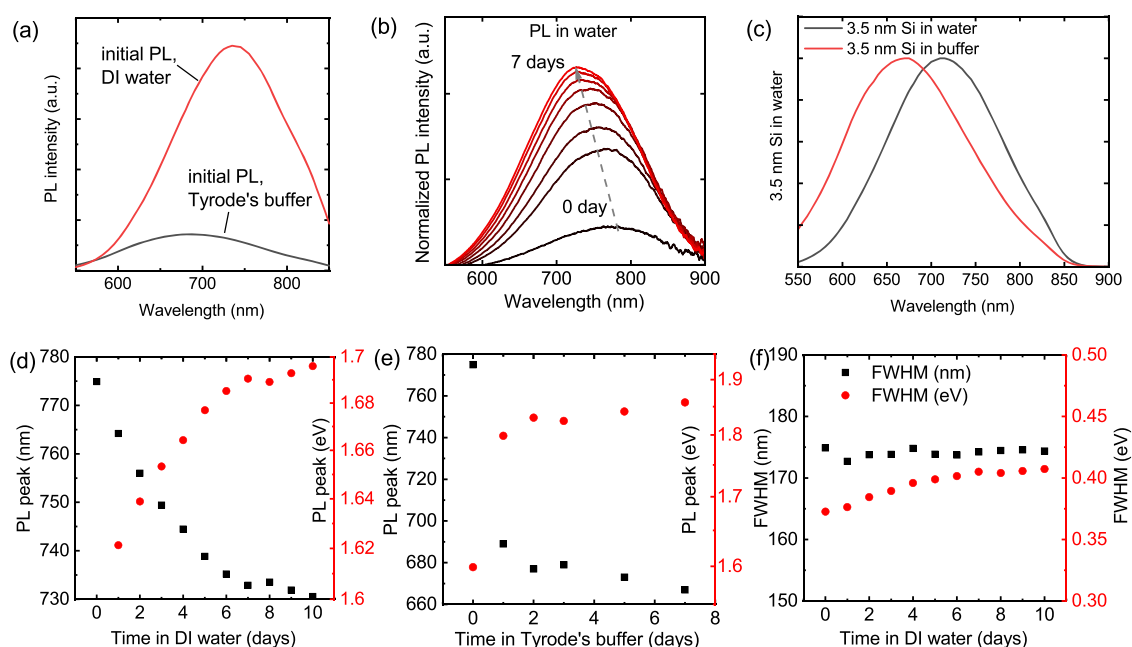


Figure 5. (a) Initial photoluminescence spectra of PEGylated AA-Si NCs in water and Tyrode's buffer. (b) Photoluminescence spectra of PEG-Si NCs dispersed in water for 0 to 7 days. The spectra are normalized with respect to the absorption measured in an integrating sphere. Photoluminescence quantum yield (c) and peak wavelength (d,e) of PEGylated AA-Si NCs dispersed in water or buffer for 0 to 10 days. (f) Evolution of the full width at half-maximum (fwhm) in water.

NCs that was oxidized in water for 7 days and then redispersed in Tyrode's buffer. These aged particles have a hydrodynamic diameter of 36 ± 7 nm in water and show 30% PLQY at 713 nm. Upon redispersing these aged particles in Tyrode's buffer, the particles form a clear dispersion with a hydrodynamic diameter of 31 ± 6 nm (Figure 3a). The PLQY dropped to 14%, which is a 53% reduction compared to that in water, while the emission peak and shape remain similar (Figure 6a).

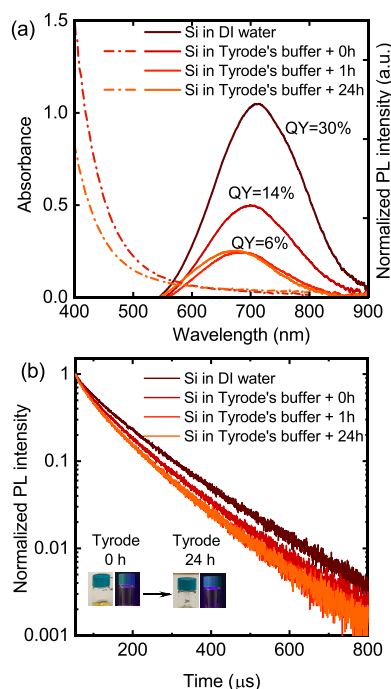


Figure 6. Degradation of 7-day oxidized PEG-Si NCs in Tyrode's buffer at 22 °C. (a) Absorption and photoluminescence spectra and (b) time-resolved photoluminescence of PEG-Si NCs before and after dispersion in Tyrode's buffer for 0, 1, and 24 h, respectively. The inset shows a picture of the sample in Tyrode's buffer under ambient light and UV irradiation.

This result indicates that PL quenching from ions is still substantial regardless of a surface oxide layer. After an hour of dispersion, the PLQY is further reduced to 6%, and this value remained constant until 24 h after dispersion. While the PLQY remains similar during the 1 to 24 h period, the total number of effective Si NC emitters decreased, as is shown by the reduced absorbance in Figure 6a and the solution color shown in the Figure 6b inset. However, the concentration of emitters is still substantial as indicated by the absorbance after 24 h. Time-resolved photoluminescence (TRPL) measurements also support that the quenching of PL occurs at a relatively fast time scale, while the number of effective emitters continues to drop after 1 h. The decay lifetime τ and dispersion factor β reduced from $\tau = 91$ μ s, $\beta = 0.82$ in water to $\tau = 75$ μ s, $\beta = 0.79$ upon dispersion into Tyrode's buffer, while the parameters after 1 and 24 h remains similar, being $\tau = 64.7$ μ s, $\beta = 0.77$ and $\tau = 64.5$ μ s, $\beta = 0.77$, respectively. The DLS, PLQY, UV-vis, and TRPL measurements suggest that these PEG-Si NCs remain colloidally stable and emitting for at least 24 h, which is attractive for bioimaging applications.

The above-described synthesis and functionalization scheme can be conveniently adjusted to achieve size-tuning. While Si NCs with 3 nm crystalline core were primarily characterized, Si

NCs with a 4 nm core size were also synthesized and investigated. Similarly, a transparent solution can be formed in water and buffer, with hydrodynamic diameters of 23 ± 6 nm and 31 ± 7 nm, respectively (Figure S4). During 7 days of dispersion in water, the PLQY increased from 2% at 870 nm to 26% at 838 nm (Figure S5).

The acrylic acid grafted Si NCs after PEGylation exhibit excellent dispersibility and red photoluminescence in a biological buffer. These characteristics indicate that PEGylated acrylic acid grafted Si NCs would be suitable for bioimaging applications if they have low cytotoxicity. Hence, we evaluated the cell-level toxicity of PEGylated AA-Si NCs using the MDA-MB-231 cell line. Figure 7 shows the cell viability for cells

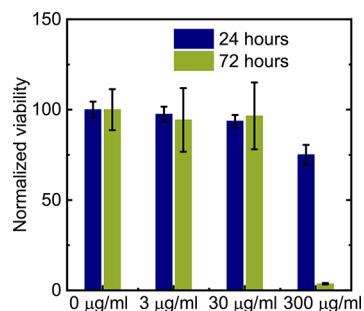


Figure 7. Viability of MDA-MB-231 cells exposed to PEGylated AA-Si NCs at 0, 3, 30, and 300 μ g/mL after 24 and 72 h.

incubated with Si NCs at three concentrations after 24 and 72 h, respectively. For Si NC concentrations of 3 μ g/mL and 30 μ g/mL, the cell viability remains higher than 90% after both 1-day and 3-day incubation. For the highest concentration, 300 μ g/mL, the cell viability is at $\sim 70\%$ after 24 h. However, after 72 h of incubation, significant cell death is observed with the cell viability $\leq 5\%$. These results were further corroborated using the bright-field images (Figure S6). As shown in Figure S6, after 72 h of incubation, high numbers of live and healthy cells can be observed at both 3 and 30 μ g/mL concentrations, whereas for the highest concentration, 300 μ g/mL, most of the cells are observed to be dead. These results indicate that the MDA-MB-231 are tolerant of PEGylated AA-Si NCs up to at least 30 μ g/mL for relatively long-time exposure (72 h). At concentrations between 30 to 300 μ g/mL, the cells can possibly retain relatively high viability under shorter-term exposure to Si NCs (~ 24 h).

The toxicity of Si NCs is highly dependent on their surface group, surface charges, and oxidative stress.^{49,50} The measured toxicity concentration threshold (30 to 300 μ g/mL) for PEGylated AA-Si NCs is consistent with that from a recent investigation on amphiphilic Si NCs using PEG surface functionalization³⁶ but lower compared to Si NCs before surface functionalization.⁵¹ The toxicity concentrations correspond to an IC_{50} value of $>10^{-4}$ nM, where IC_{50} is defined as the exposure concentration for 50% cell death after 24 h. Compared with previous values reported in the literature, this IC_{50} value is about 80% higher than that of cadmium-containing quantum dots and other quantum dots,⁵² demonstrating that the acrylic acid grafted and PEGylated Si NCs are relatively nontoxic. It should be noted that these studies were performed using different cell types, cell concentrations, and assay types, which can all affect the results of toxicity evaluation.

CONCLUSIONS

We have successfully prepared NIR emitting, water, and Tyrode's buffer dispersible Si NCs through a combination of gas phase and colloidal synthesis. The acrylic acid grafted and PEGylated Si NCs exhibit PL with a maximum peak wavelength at ~ 700 nm and PLQYs of $\sim 30\%$ and $\sim 14\%$ in water and Tyrode's buffer, respectively. Compared to other synthetic approaches, our entire synthetic process occurs at room temperature with minimal amount of solvent and without additional postsynthetic steps or catalysts. While we demonstrate that acrylic acid grafted Si NCs can be synthesized in an all-gas-phase setup on a time scale of 10 ms, these particles exhibit low stability and negligible PL in biological buffer. Nevertheless, the acrylic acid grafted Si NCs enable better dispersibility in methanol and undergo PEGylation under room temperature to form stable dispersions in water and Tyrode's buffer with NIR emission, which makes them a worthy candidate for biological imaging. The cell toxicity of acrylic acid grafted Si NCs after PEGylation is evaluated with the MDA-MB-231 cell line, and it is found that the MDA-MB-231 are tolerant of PEGylated AA-Si NCs up to at least $30 \mu\text{g/mL}$ for 72 h.

EXPERIMENTAL SECTION

Synthesis of Acrylic Acid Grafted Si NCs. Acrylic acid functionalized Si NCs were synthesized in a nonthermal plasma reactor similar to the ones in previous works.^{27,53} The reactor consists of a 0.5 cm inner diameter borosilicate tube which expands to 2 cm. Ten to thirty standard cubic centimeters per minute (sccm) of argon and 14 sccm of silane (5% in helium) mixture were flowed through the tube, while the acrylic acid was injected as vapor through a bubbler into the plasma afterglow. Fifty standard cubic centimeters per minute of argon was used as the carrier gas for the bubbler, and the bubbler pressure was held at ~ 25 Torr. The plasma was generated by applying radio frequency (13.65 MHz) power to a pair of copper ring electrodes with a nominal power of 60 W. The powered electrode was fixed at 3–4 cm upstream of the acrylic acid injection port unless specified otherwise. The pressure in the plasma region was held at ~ 1.4 Torr. Particles were collected downstream of the plasma using a fine stainless steel mesh.

PEGylation of Acrylic Acid Grafted Si NCs. Acrylic acid functionalized Si NCs collected for 30 min were dispersed in a mixture of 0.5 mL polyethylene glycol (PEG-OH) 400 (BioChemica, molecular weight 380–420) and 5 mL methanol. By bypassing acrylic acid injection during the synthesis, we estimated the mass of the core Si NCs to be ~ 5 mg. The nanoparticles formed a clear dispersion in the PEG-OH/methanol mixture by briefly shaking the container by hand. Methanol was then evaporated by flowing nitrogen over the solution, and the AA-Si NCs and PEG-OH mixture (5 mg Si NCs:0.5 mL PEG-OH) were kept at room temperature for 72 h. For purification, 1 mg Si NCs was dispersed in 0.5 mL DI water, and excess PEG was removed using an Amicon 3 kDa MWCO centrifuge filtration unit (Sigma-Aldrich, Z677094) at 12000 rpm for 60 min and repeated twice. The Si NCs were then redispersed in 0.2 mL methanol prior to FT-IR measurements.

Si NC Characterization. The nanoparticles were characterized with FT-IR, XPS, zeta potential measurements, DLS, and TEM. FT-IR was performed with a Bruker Alpha spectrometer with the attenuated total reflection (ATR) single reflective module in a nitrogen-filled glovebox. Samples dissolved in methanol were drop-cast and allowed to dry on the ATR crystal, and 20 scans were taken for each measurement at 2 cm^{-1} resolution. XPS was taken with a PHI Versa Probe III XPS and ultraviolet photoelectron spectroscopy (UPS) system. Nanoparticles were drop-cast and allowed to dry to form a film. Zeta potential measurements were taken with a Stabino Zeta potential analyzer.

PLQY was measured with an integrating sphere setup that was previously described.⁵⁴ The excitation source is a LED centered at 395 nm, and the PL spectra were collected by an OceanOptics USB2000 spectrometer calibrated to an OceanOptics HL-3 Plus lamp. TRPL measurements were taken with a Horiba DeltaFlex TCSPC Lifetime Fluorometer. The excitation source is a 407 nm laser with a delay time of 13 μs .

UV–vis measurements were carried out with a Cary 7000 UV–vis spectrophotometer.

Toxicity of PEGylated AA-Si NCs. The toxicity of PEGylated AA-Si NCs was evaluated using the MDA-MB-231 cell line and Cell Counting Kit 8 (WST-8, Abcam). MDA-MB-231 cells were cultured in a transparent 96-well plate (ThermoFisher) at 7500 cells per well. The plate was then placed in a 37 °C incubator with 5% CO_2 . After 18 h, media was removed from each well, and 100 μL media containing the appropriate concentration of Si NCs, or without Si NCs as the control group, was added to the wells. The cells were then incubated with the Si NCs for 1 and 3 days. After 24 h, 10 μL of WST-8 solution was added to the wells. After 4 h of incubation in a 37 °C incubator, the absorbance at 460 nm was measured for those wells. The plate was then returned to a 37 °C incubator. After 72 h, the cells were tested again where the WST-8 solution was added to the remaining wells, and absorbance was measured after 4 h of incubation.

The absorbance was measured with a TECAN Infinite M200 spectrophotometer and normalized against the values for media with just Si NCs. Cell viability was determined using the ratio of absorbances for experimental conditions (cells with Si NCs) to cells with media only.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsabm.1c00885>.

Additional characterization, including XRD, XPS, DLS of the acrylic acid grafted, 3 nm Si NCs, and TEM, and PL evolution of 4 nm Si NCs, and bright-field microscopic images of MDA-MB-231 cells incubated with PEGylated AA-Si NCs.

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Notes

The authors declare no competing financial interest.

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