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Photoresponsive Real Time Monitoring Silicon Quantum Dots for Regulated Delivery of Anticancer Drug

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Recently, photoresponsive nanoparticles are widely used to develop drug delivery systems (DDSs) wherein light is used as an external stimulus to trigger drug release in spatially and temporally controlled fashion. Real time monitoring DDSs are also gaining much interest due to their capability of monitoting drug release in situ. In this context we designed a new photoresponsive real time monitoring nanoparticle based on photoluminescent Silicon Quantum Dots (SiQDs) using o-nitrobenzyl (ONB) derivative as phototrigger for the controlled release of anticancer drug chlorambucil (Cbl). The strong fluorescence of SiQDs was initially quenched by ONB. Upon irradiation ONB triggered the release of drug causing switching on the fluorescence of SiQDs to monitor the drug release. We reported a new and simple strategy to synthesise amine functionalised silicon quantum dots and covanlently conjugated phototrigger ONB with caged anticancer drug Cbl on to it. Newly designed photoresponsive theranostic ONBCbl-SiQDs performed three important functions: (i) nanocarrier for drug delivery, (ii) controlled drug release under both one photon and two-photon excitation, and (iii) photoswitchable fluorescent nanoparticles for real-time monitoring of drug release based on photoinduced electron transfer (PET) process. In vitro biological studies revealed efficient cellular internalisation and cancer cell destruction ability of ONBCbl-SiQDs on photoirradiation. ONBCbl-SiQDs exhibit a successful example of combining multiple functions into a single system for drug delivery system.

Introduction

Photoresponsive nano drug delivery systems (DDSs) combining both therapeutic and diagnostic functionalities in a single system have captured great attention because of their key ability to regulate drug release spatially and temporally by externally regulated light.[1] In general, photoresponsive nano DDSs consist of two main components, namely, biocompatible nanoparticles and a small organic molecule "phototrigger".[2] On the other hand real time monitoring DDS also attracting utmost interest for drug delivery because they are capable of monitoring the drug delivery location and delivered amount *in situ*,[3-6] allowing to determine the drug delivery kinetics. Recently real time monitoring of drug delivery from DDSs has been performed through Forster resonance energy transfer (FRET),[7] aggregation induced emission (AIE),[8] or a quenching system,[9] consisting of a fluorophore or nanoparticles. By any means, if we can develop single component photoresponsive real time monitoring drug delivery system, it will be helpful to maximize control over drug release and minimize undesirable side effects.

Recently, photoluminescent silicon quantum dots (SiQDs) have emerged as promising material in the area of cellular imaging. SiQDs are well explored as *in vitro*[10-12] and *in vivo*[13,14] imaging agents because of their unique optical properties, high natural abundance, low toxicity, excellent surface tailorability, size dependent tunable emission, strong fluorescence and low photobleaching properties.[15] Drug conjugation to SiQDs and controlled release of the loaded drug in response to the pH change have been reported recently.[16-18] The aforesaid unique features of SiQDs have inspired us to design a photoresponsive nano DDS based on SiQDs for the first time. Here SiQDs are used as biocompatible fluorescent nanocarriers and *o*-nitrobenzyl (ONB) as phototrigger for controlled release of anticancer drug, chlorambucil. In this study, we used *o*-nitrobenzyl (ONB) moiety as a phototrigger for two reasons i) phototrigger to cage the anticancer drug chlorambucil and ii) nitrobenzene derivatives are well known fluorescent quenchers as they decrease the LUMO level of the molecules, making them excellent acceptor in photoinduced electron

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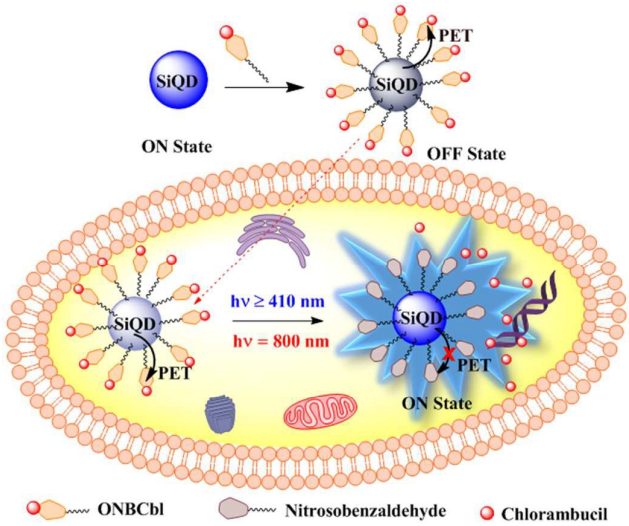
† Electronic Supplementary Information (ESI) available: Synthetic scheme of ONBCbl, ^{1}HNMR, ^{13}CNMR spectra, EDX pattern of ONBCbl-SiQDs, quantum yield calculations, HPLC profile and other experimental details. See DOI: 10.1039/x0xx00000x

transfer (PET) process inducing fluorescence quenching. Initially *o*-nitrobenzyl (ONB) moiety with caged anticancer drug chlorambucil quenches the fluorescence of SiQDs due to PET process and this state is known as fluorescence "off" state. Upon irradiation uncaging of the drug occurred and this led to the switching on of the fluorescence of SiQDs, consider as fluorescence "on" state **(Scheme 1)**. This phenomenon was utilised for the real time monitoring of the drug release. Moreover, ONB moiety has high photochemical quantum yield with very fast and efficient release ability upon both one-photon (1PE) and two photon (2PE) excitation[19] which could be further useful in releasing the drug deep inside the body.



Scheme 1 Schematic representation of controlled release and real time monitoring of anticancer drug chlorambucil from ONBCbl-SiQDs.

Experimental Section

Materials and Method

All reagents were purchased from Sigma Aldrich and used without further purification. Toluene was distilled using sodium and benzophenone and dichloromethane (DCM) was distilled using CaH_2 before use. ^{1}H NMR spectra were recorded on a BRUKER-AC 200 MHz spectrometer. Chemical shifts are reported in ppm from tetramethylsilane with the solvent resonance as the internal standard (deuterochloroform: 7.26 ppm). Data are reported as follow: chemical shifts, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constant (Hz). ^{13}C NMR (50 MHz) spectra were recorded on a BRUKER-AC 200 MHz Spectrometer with complete proton decoupling. Chemical shifts are reported in ppm from tetramethylsilane with the solvent resonance as the internal standard (deuterochloroform: 77.23 ppm). UV/vis absorption spectra were recorded on a Shimadzu UV-2450 UV/vis spectrophotometer, fluorescence emission spectra were recorded on a Hitachi F-7000 fluorescence spectrophotometer, FTIR spectra were recorded on a Perkin Elmer RXI spectrometer. Transmission Electron Microscopy (TEM) was measured on a FEI Tecnai G220S-Twin at 200 kV. The TEM sample was prepared by dispersing compounds in methanol and dropping on the surface of a copper grid. Photolysis of all the ester conjugates were carried out using 125 W medium pressure Hg lamp supplied by SAIC (India). Chromatographic purification was done with 60-120 mesh silica gel (Merck). For reaction monitoring, precoated silica gel 60 F254 TLC sheets (Merck) were used. Revearse phase HPLC (RP-HPLC) was taken using mobile phase acetonitrile, at a flow rate of 1 mL/min (detection: UV 254 nm).

Synthesis of 4-(3-bromopropoxy)5-methoxy-nitrobenzaldehyde[20]

1,3 dibromopropane (3.98 g, 2.01 mL, 19.71 mmol) was added to a solution of vanillin (1 g, 6.57 mmol) in DMF (2 mL), containing K_2CO_3 (1.18 g, 8.54 mmol). The reaction was then stirred at 80 °C for 1 h. Subsequently the reaction mixture was cooled to room temperature, diluted with ethyl acetate and washed many times with brine (200 mL) and the organic layer was dried over Na_2SO_4. The crude material was purified by column chromatography through silica gel using EtOAc/hexane (1: 2) to yield 954 mg (3.49 mmol, 53 %) of product as a white solid. ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 9.83 (1H, s), 7.45-7.41 (1H, d, J = 8.2 Hz), 7.39 (1H, s,), 7.02-6.98 (1H, d, J = 8.2 Hz), 4.25-4.19 (2H, t, J = 6 Hz), 3.89 (3H, s), 2.42-2.36 (2H, q, J = 6 Hz) ; ^{13}C NMR ($CDCl_3$, 50 MHz): δ =190.9 (1C), 153.8 (1C), 150.0 (1C), 130.41 (1C), 126.7 (1C), 111.9 (1C), 109.6 (1C), 66.6 (1C), 56.1 (1C), 32.1 (1C), 29.8 (1C)

Synthesis of 4-(3-bromopropoxy)-5-methoxy-2-nitrobenzaldehyde[21]

4-(2-Bromoethoxy)-3-methoxybenzaldehyde (900 mg, 3.3 mmol) was slowly added to 20 mL of ice-cold conc. HNO_3. The temperature of the solution was slowly raised to room temperature, and the reaction was stirred for 1 h. The reaction mixture was then poured onto ice water and the precipitate was filtered off then washed with cold water. The product was recrystallized from boiling EtOH (approximately 30 mL), giving 4-(3-bromopropoxy)-5-methoxy-2-nitrobenzaldehyde in 97 % yield as a yellow solid (1.02 g, 3.2 mmol). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 10.42 (1H, s), 7.63 (1H, s), 7.39 (1H, s), 4.32-4.26 (2H, t, J = 5.8 Hz,), 3.99 (3H, s), 3.66-3.59 (2H, t, J = 6.2 Hz), 2.45-2.39 (2 H, q, J = 6 Hz). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 190.0 (1C), 153.7 (1C), 151.7 (1C), 143.9 (1C), 125.9 (1C), 110.1 (1C), 108.4 (1C), 67.3 (1C), 56.8 (1C), 31.9 (1C), 29.5 (1C).

Synthesis of 4-(3-bromopropoxy)-5-methoxy-2-nitrophenyl) methanol[22]

4-(3-bromopropoxy)-5-methoxy-2-nitrobenzaldehyde (1 g, 3.1 mmol) was dissolved in 20 mL of methanol and made ice cold. To the ice cold solution $NaBH_4$ was added slowly and the resultant solution was stirred at room temperature for 1 h. The solvent was then evaporated, and the residue was partitioned between EtOAc and water. After extraction with EtOAc, organic layers was washed with brine, dried over Na_2SO_4, filtered, and evaporated. Recrystallization from EtOAc gave 4-(3-bromopropoxy)-5-methoxy-2-nitrophenyl)methanol (908 mg, 2.8 mmol) as a yellow solid and the yield was 91 %. ^{1}H

NMR ($CDCl_3$, 200 MHz): δ = 7.69 (1H, s), 7.17 (1H, s), 4.92 (2H, s), 4.22-4.16 (2H, t, J = 6 Hz), 3.95 (3H, s), 3.64-3.58 (2H, t, J = 6 Hz), 2.40-2.34 (2H, q, J = 6 Hz). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 154.5 (1C), 147.1 (1C), 139.6 (1C), 132.9 (1C), 111.2 (1C), 109.9 (1C), 67.1 (1C), 62.8 (1C), 56.5 (1C), 32.1 (1C), 29.8 (1C).

Synthesis of 1-(bromoethyl)-4-(3-bromopropoxy)-5-methoxy-2-nitrobenzene[23]

4-(3-bromopropoxy)-5-methoxy-2-nitrophenyl)methanol (847 mg, 2.6 mmol) to the stirred solution of PBr_3 (716.2 mg, 0.25 mL , 2.8 mmol) in CH_2Cl_2 (20 mL) under N_2 atmosphere with external cooling. The solution was stirred at room temperature for 1 h and then refluxed for 2 h. The reaction mixture was then poured into H_2O (50 mL) and was neutralized with a 2 N aqueous KOH solution (100 mL). The organic phase was then separated, and the aqueous phase was rinsed twice with CH_2Cl_2 (50 mL). The solution was then dried over Na_2SO_4 (5 g) and the solvent was removed to yield 1-(bromoethyl)-4-(3-bromopropoxy)-5-methoxy-2-nitrobenzene (766 mg, 2 mmol, 77 %) as dirty yellow solid. ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 7.68 (1H, s), 6.93 (1H, s), 4.85 (2H, s), 4.24-4.18 (2H, t, J = 6 Hz), 3.97 (3H, s), 3.64-3.58 (2H, t, J = 6 Hz), 2.41-2.35 (1H, q, J = 6 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 153.6 (1C), 148.1 (1C), 140.1 (1C), 127.7 (1C), 113.9 (1C), 110.0 (1C), 66.9 (1C), 56.5 (1C), 31.9 (1C), 30.1 (1C), 29.5 (1C).

Synthesis of 4-(3-bromopropoxy)-5-methoxy-2-nitrobenzyl chlorambucil (ONBCbl)

Chlorambucil (100 mg, 0.32 mmol) was dissolved in 1 mL dry DMF, to it K_2CO_3 (54 mg, 0.39 mmol) and the resultant solution was stirred for 20 min at room temperature. Then to the reaction mixture 1-(bromoethyl)-4-(3-bromopropoxy)-5-methoxy-2-nitrobenzene (126 mg, 0.32 mmol) was added and the resultant mixture was stirred for 8 h at room temperature. Then the reaction mixture was diluted with ethyl acetate and washed many times with brine (200 mL) and the organic layer was dried over Na_2SO_4. The crude product was purified by column chromatography through silica gel using EtOAc/hexane (1:1) to yield 100 mg (0.16 mmol, 50%) of 4-(3-bromopropoxy)-5-methoxy-2-nitrobenzyl chlorambucil as a dark red liquid.^{1}H NMR ($CDCl_3$, 200 MHz): δ = 7.73 (1H, s), 7.07-7.03 (2H, d, J = 8.6 Hz), 6.99 (1H, s), 6.63-6.59 (2H, s, J=8.6 Hz), 5.47 (2H, s), 4.24-4.18 (2H, t, J = 6 Hz), 3.92 (3H, s), 3.71-3.68 (4H, t, J = 6 Hz), 3.66-3.63 (4H, t, J = 6 Hz), 3.61-3.58 (2H, t, J = 6 Hz), 2.61-2.54 (2H, t, J = 6 Hz), 2.46-2.41 (2H, t, J = 6 Hz), 2.41-2.35 (2H, t, J = 6 Hz), 1.98-1.91(2H, m), ^{13}C NMR ($CDCl_3$, 50MHz): δ = 172.9 (1C), 153.9 (1C), 147.5 (1C), 144.5 (1C), 140.0 (1C), 130.4 (1C), 129.8 (1C), 127.4 (1C), 112.3 (1C), 110.9 (1C), 109.9 (1C), 67.0 (1C), 63.2 (1C), 56.4 (1C), 53.6 (1C), 40.6 (1C), 34.0 (1C), 33.6 (1C), 32.0 (1C), 29.7 (1C), 26.9 (1C)

Synthesis of silicon quantum dots（SiQDs)

At first $SiCl_4$ (0.5 mL, 2.9 mmol) was dissolved in the solution of cetyl trimethylammonium bromide (CTAB) (3.2 g) and anhydrous toluene (100 mL) and the solution was stirred at room temperature for 15 min at room temperature. $LiAlH_4$ (0.45 g, 11.8 mmol) was added to the resultant solution and it was stirred for 3 h at room temperature. Then anhydrous methanol (20 mL) was slowly added to quench any excess $LiAlH_4$ after that the solvent was removed to yield silicon quantum dots as white solid. Silicon quantum dots thus formed had hydrogen terminated surface. The surface of SiQDs were then functionalised with amine functional group by suspending hydrogen terminated SiQDs (300 mg) and (3-aminopropyl)triethoxysilane (APTS) (1.4 g) in dry toluene and refluxing the resultant mixture for 12 h. After modification of the surface, the sample was purified in a centrifuge (7000 rpm min^{-1}, 20 min) to yield (787 mg) amine functionalised SiQDs as white solid.

Synthesis of 4-(3-bromopropoxy)-5-methoxy-2-nitrobenzyl chlorambucil (ONBCbl) loaded silicon quantum dots (ONBCbl-SiQDs)

SiQDs (100 mg) were dispersed in dry tetrahydrofuran (THF) (3 mL) under a N_2 atmosphere and potassium tertiary butoxide (75 mg) was added. After stirring for 1 h at room temprature, 4-(3-bromopropoxy)-5-methoxy-2-nitrobenzyl chlorambucil dissolved in dry THF was added dropwise to the mixture and the resulting solution was stirred for 24 h at room temperature in the dark. Finally *o*-nitrobenzyl chlorambucil loaded SiQDs (ONBCbl-SiQDs) was purified by centrifuging (7000 rpm min^{-1}, 20 min) yielding 90 mg product.

Photolysis of ONBCbl-SiQDs using soft visible light irradiation (≥ 410 nm)

These experiments were carried out using a previously described method. 1 mg of ONBCbl-SiQDs was dissolved in 1 mL of acetonitrile in quartz cuvettes. They were irradiated for 30 min under UV light by 125 W cm^{-2} medium pressure Hg vapour lamp using $NaNO_2$ solution (1 M) as a UV cut off filter. At regular interval of time (5 min), 15 µL of the aliquots was taken and analyzed by RP-HPLC using mobile phase acetonitrile, at a flow rate of 1 mL/min (detection: UV 254 nm). Peak areas were determined by RP-HPLC, which indicated gradual increase of the released chlorambucil with time, and the average of three runs. Based on HPLC data for each chlorambucil release, we plotted normalized [A] (HPLC peak area) versus irradiation time. We observed an exponential correlation for the appearance of the released chlorambucil, which suggested a first order reaction.

Photolysis of ONBCbl-SiQDs using diode laser (800 nm)

1 mg of ONBCbl-SiQDs was dissolved in 1 mL of acetonitrile. Half of the solution was kept in dark and to the remaining half nitrogen was passed and was irradiated using diode laser (800 nm, 500 mW/ cm^2). At regular time intervals, a small aliquot (100 µL) of the suspension was taken out and analyzed by reversed phase HPLC using mobile phase acetonitrile, at a flow rate of 1 mL / min.

Cell Imaging of SiQDs and ONBCbl-SiQDs using HeLa cell line

Cell imaging studies were carried out using the HeLa cell line, which was maintained in minimum essential medium (MEM)

containing 10 % FBS at 37 ˚C and 5 % CO_2. To study the cellular uptake SiQDs and ONBCbl-SiQDs, HeLa cells (5 x 10^4 cells/well) were placed on six-well plates and allowed to adhere for 4-8 h. The cells were then incubated with SiQDs and ONBCbl-SiQDs 20 µg/mL in cell culture medium at 37 ˚C and 5 % CO_2 for 6 h. Thereafter, the cells were fixed in 4 % aqueous formaldehyde for 15 min and washed two times with PBS. Imaging was captured by a Nikon confocal microscope (Nikon Eclipse TE2000-E) using the respective filter.

Cytotoxicity of SiQDs and ONBCbl-SiQDs using HeLa cell line

Cytotoxicity before photolysis:

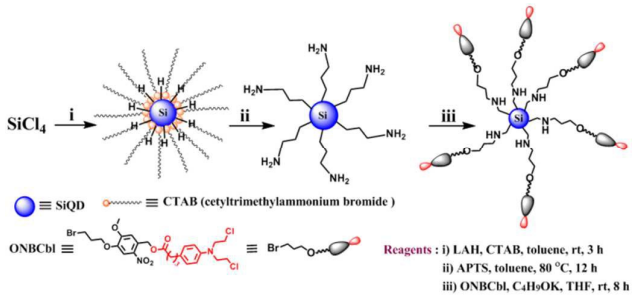
The cytotoxicity *in vitro* was measured using the MTT assay on the HeLa cell line. Briefly, cells growing in log phase were seeded into a 96-well cell culture plate at 1x10^4 cells/mL. SiQDs, ONBCbl-SiQDs and chlorambucil were added into the wells with an equal volume of PBS in the control wells. The cells were then incubated at 37 ˚C in 5 % CO_2 for 48 h. Thereafter, fresh media containing MTT (0.40 mg mL^{-1}) were added to the 96-well plate and incubated at 37 ˚C in 5 % CO_2 for an additional 4 h. Formazan crystals formed were dissolved in DMSO after decanting the media, and then absorbance was recorded at 595 nm.

Cytotoxicity after photolysis

HeLa cells in a 96-well cell culture plate at a concentration of 1x10^4 cells/mL were maintained in MEM containing 10 % FBS. SiQDs (0.1-50 µg/mL), ONBCbl-SiQDs (0.1-50 µg/mL) and chlorambucil (0.1- 50 µM), with different concentrations were incubated at 37 ˚C and 5 % CO_2 for 48 h. Then, the cells were irradiated for 30 min using a 125 W cm^{-2} medium-pressure Hg lamp as the irradiation source (≥410 nm) with $NaNO_2$ solution (1 M) as a UV cut off filter by keeping the cell culture plate 5 cm away from the light source. After irradiation, the cells were incubated for another 48 h. Finally, the cytotoxicity was measured using the MTT assay as described in the previous section.

Results and discussion

Synthesis and Characterisation of *o*-nitrobenzyl-chlorambucil conjugated silicon quantum dots (ONBCbl-SiQDs)



Scheme 2 Synthesis of *o*-nitrobenzyl chlorambucil tagged SiQDs as photoresponsive DDS.

We presented a new synthetic approach to synthesize amine functionalised silicon quantum dots (SiQDs) by modifying the existing synthetic procedure.[24] Commercially available silicon tetrachloride ($SiCl_4$) was reduced with lithium aluminum hydride ($LiAlH_4$) in presence of surfactant cetyltrimethylammonium bromide (CTAB) in dry toluene at room temperature for 3 h. Hydrogen-terminated silicon quantum dots (Si-H) thus produced were then functionalised with amine terminals by reacting with (3-aminopropyl)triethoxysilane (APTS) in dry toluene at 80 ˚C for 12 h thereby forming SiQDs. Photocaged anticancer drug, 4-(3-bromopropoxy)-5-methoxy-2-nitrobenzyl chlorambucil (ONBCbl) was synthesized separately, as shown in **Scheme S1 ESI†** in the Supporting Information. Finally, the ONBCbl was covalently attached on the surface of SiQDs using potassium tert-butoxide as base in dry THF **(Scheme 2)**.

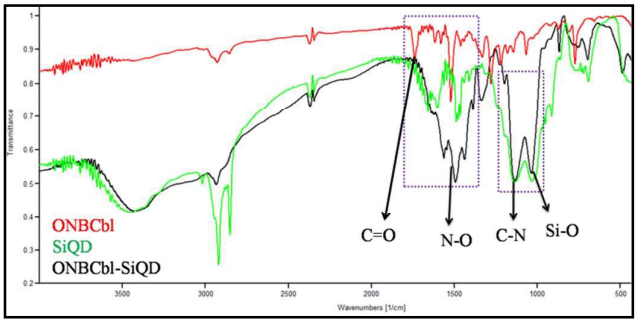


Fig. 1 FTIR spectra of *o*-nitrobenzylchlorambucil (ONBCbl), silicon quantum dot (SiQDs) and *o*-nitrobenzyl chlorambucil loaded silicon dots (ONBCbl-SiQDs).

Surface functionalization of SiQDs and attachment of ONBCbl to the surface of SiQDs was monitored by FTIR spectroscopy **(Fig. 1)**. SiQDs has a broad peak at 3100–3500 cm^{-1} which corresponds to the stretching vibration of O-H and N-H, peaks at 1035 cm^{-1} and 1140 cm^{-1} correspond to the stretching vibration of Si-O and C-N respectively, peaks at 2930 cm^{-1} and 2860 cm^{-1} correspond to aliphatic CH_2 due to APTS assisted surface modification of SiQDs. In the FTIR spectrum of ONBCbl-SiQDs a new peak at 1500 cm^{-1} corresponding to N-O stretching frequency of NO_2 appeared which indicates that ONBCbl is successfully attached to the surface of SiQDs. We also carried out energy dispersive X-Ray (EDX pattern) and elemental mapping of Si, C, O, N, and Cl for ONBCbl-SiQDs **(Fig. 2c)**. The images clearly validate the homogenous distribution of Si, C, O, N, and Cl elements throughout the material. EDX pattern further describes the presence of the corresponding constituting elements **(Fig. S1, ESI†)**.

Size and shape of the NH_2 functionalised silicon quantum dots (SiQDs), and ONBCbl conjugated silicon quantum dots (ONBCbl-SiQDs) were determined by TEM and DLS analysis. TEM of SiQDs and ONBCbl-SiQDs are presented in **Fig.2a** and **b** respectively, they show that SiQDs and ONBCbl-SiQDs were spherical in shape and their average diameters were ~6-10 nm and ~20-30 nm respectively. Dynamic light scattering (DLS) study further revealed that the hydrodynamic diameters of

SiQDs and ONBCbl-SiQDs were 6.7 ± 1.6 nm and 26.4 ± 1 nm **(Fig. 3a** and **b)**, respectively. The size of the ONBCbl-SiQDs were within the preferred range of nanoparticles that can be used for drug delivery.[25] ONBCbl-SiQDs were larger in size compared to free SiQDs, which clearly indicated that ONBCbl was anchored on the surface of SiQDs.

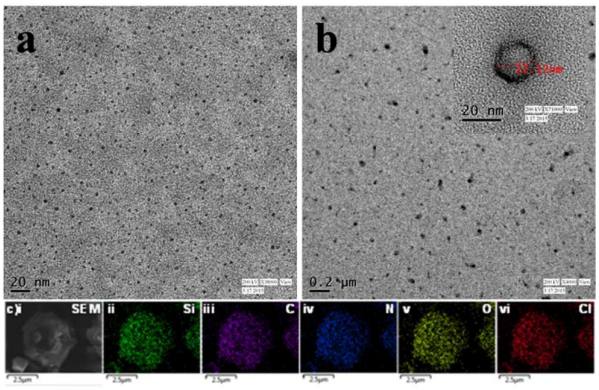


Fig. 2 TEM image of (a) silicon quantum dots (SiQDs), (b) ONBCbl conjugated silicon quantum dots (ONBCbl-SiQDs), (c) SEM X-ray elemental mapping of ONBCbl-SiQDs showing the presence of constituting elements: (i) SEM image, (ii) Silicon (Si), (iii) Carbon (C), (iv) Nitrogen (N), (v) Oxygen (O) and, (vi) Chlorine (Cl).

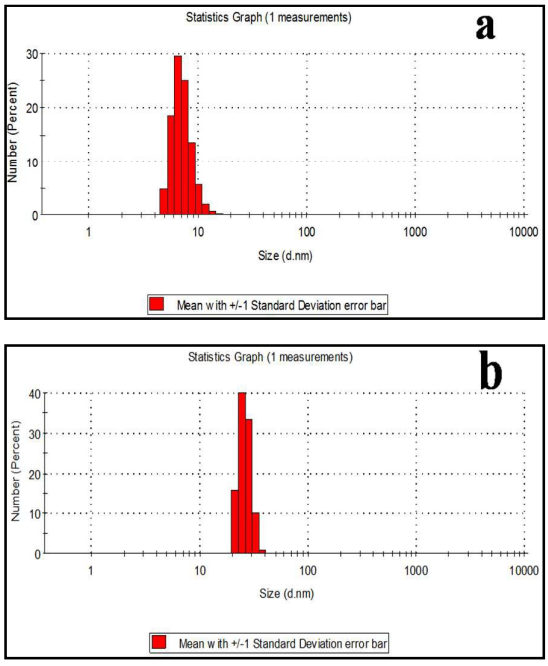


Fig. 3 Particle size distribution graph of (a) SiQDs, (b) ONBCbl-SiQDs as revealed by DLS.

The total amount of ONBCbl loaded on the surface of SiQDs was determined by UV-Vis absorption spectra **(Fig. S2 a, b** and **c, ESI†)**, it was calculated to be about 22 µg/mg of SiQDs.

Photophysical Properties of ONBCbl-SiQDs and SiQDs

Further, photophysical properties of ONBCbl-SiQDs were studied. UV-Vis spectrum of ONBCbl-SiQDs dispersed in acetonitrile showed a broad absorbance band from 300 nm to 475 nm **(Fig. 4a)**. It can be noted that free SiQDs do not have such broad absorption band. SiQDs dispersed in acetonitrile exhibited strong blue emission at 430 nm, when excited at 360 nm, but the fluorescence of SiQDs got quenched upon conjugation with ONBCbl **(Fig. 4b)**. This phenomenon can be explained by the Photoinduced Energy Transfer (PET) process from SiQDs to ONBCbl which was due to the presence of electron withdrawing group (nitro group) in the phototrigger **(Scheme 3)**. Fluorescence quantum yields of SiQDs and ONBCbl-SiQDs are 18.8 % and 4.2 %, respectively (quinine sulphate quantum yield: 54 %)[26] as shown in **Table S1 ESI†**. Hence ONBCbl-SiQDs can be utilized for cellular imaging along with real time monitoring of drug release.

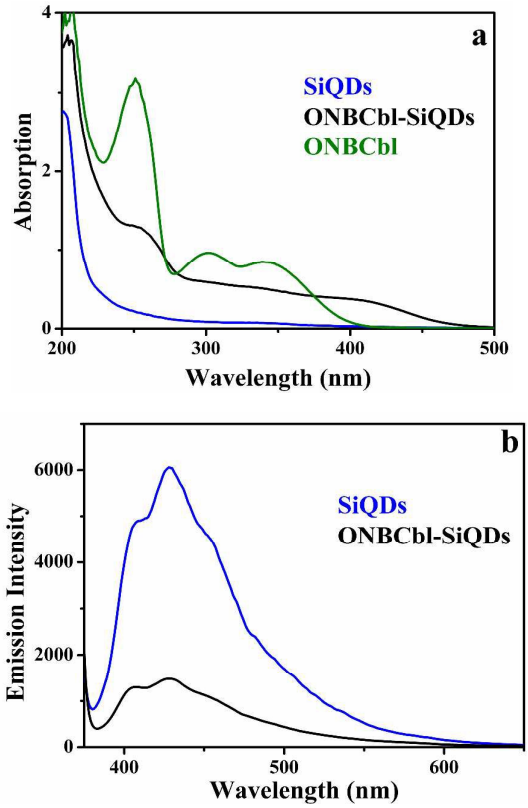


Fig. 4 (a) UV-Vis absorption spectrum of SiQDs, ONBCbl-SiQDs, and ONBCbl and (b) emission spectrum of SiQDs and ONBCbl-SiQDs in acetonitrile.

Photolysis of Photocaged ONBCbl-SiQDs

The photoinduced release of the anticancer drug, chlorambucil from ONBCbl-SiQDs was demonstrated by irradiating the suspension of ONBCbl-SiQDs in acetonitrile under both visible light (≥ 410 nm, Hg-vapour lamp) and two-photon excitation using 800 nm, diode laser. The course of the photorelease was monitored by emission spectroscopy and reverse phase HPLC. It was observed from the emission spectra **(Fig. 5a)** that the emission intensity of the suspension of ONBCbl-SiQDs in acetonitrile steadily increased with increasing irradiation time. After 30 min photoirradiation, it was found that the

fluorescent intensity of ONBCbl-SiQDs suspension in acetonitrile changed from faint **(Fig. 5b)** to bright blue **(Fig. 5c)** which is the characteristic emission of SiQDs. This phenomenon of photoinduced fluorescence switch "off"-"on" of ONBCbl-SiQDs can be exploited for real time monitoring of the drug release. By correlating the increase in fluorescence intensity with the percentage of drug released, it was found that after 30 min of irradiation about 74 % of drug was released as shown **Fig. 5d**.

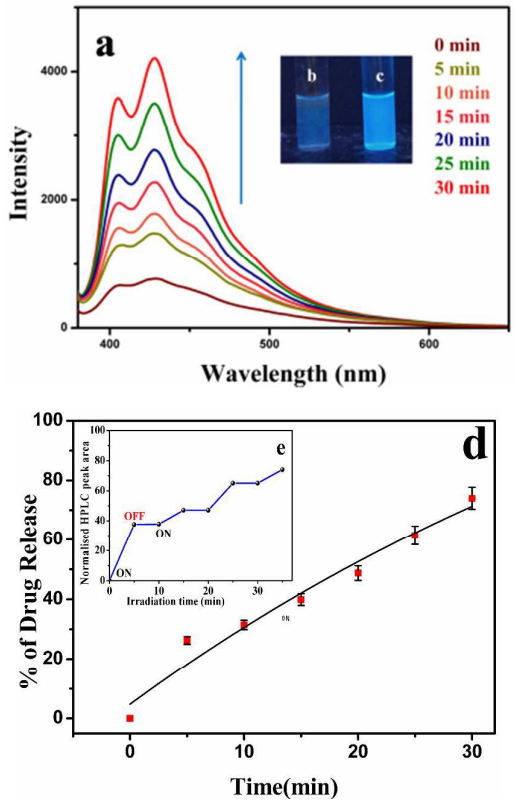
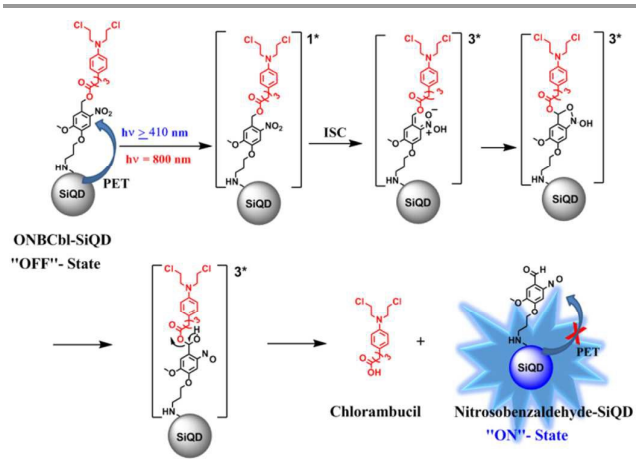


Fig. 5 (a) Emission spectra of the ONBCbl-SiQDs at different time intervals of photoirradiation (0-30 min, ≥ 410 nm), (b) fluorescence before photolysis at 0 min, (c) fluorescence after 30 min irradiation, (d) percentage of chlorambucil released as a function of fluorescence intensity change, e) the partial progress for the release of chlorambucil under bright and dark conditions. "ON" indicates the beginning of light irradiation, "OFF" indicates the ending of light irradiation.

On the basis of literature reports,[27,28] we proposed mechanism for the photorelease of anticancer drug chlorambucil from ONBCbl-SiQDs as shown in **Scheme 3.** Upon irradiation phototrigger conjugated chlorambucil (ONBCbl) undergoes photolysis to release the anticancer drug chlorambucil thereby producing nitrosobenzaldehyde-SiQDs as photoproduct **(Scheme 3)**. Energy transfer process from SiQDs was stopped in the presence of nitrosobenzaldehyde group and so SiQDs regained their inherent fluorescence after photolysis.



Scheme 3 Proposed mechanism for the photorelease of anticancer drug chlorambucil and fluorescence "turn on" ability of ONBCbl-SiQDs.

From the HPLC profile **(Fig. S3, ESI†)** it was also observed that after 30 min of irradiation (λ ≥ 410 nm, 125 W cm^{-2}) 74 % of the loaded anticancer drug chlorambucil was effectively released, whereas 12 % of the drug was released using diode laser (800 nm, 500 mW cm^{-2}) **(Fig. 6)**, this clearly indicates that the percentage of drug release is highly dependent on external light intensity. We also studied controlled photolytic release of anticancer drug from ONBCbl-SiQDs by monitoring the release processes while exposing it to light and dark conditions alternately. **Fig. 5e** (inset), shows that drug was released from ONBCbl-SiQDs only under irradiation.

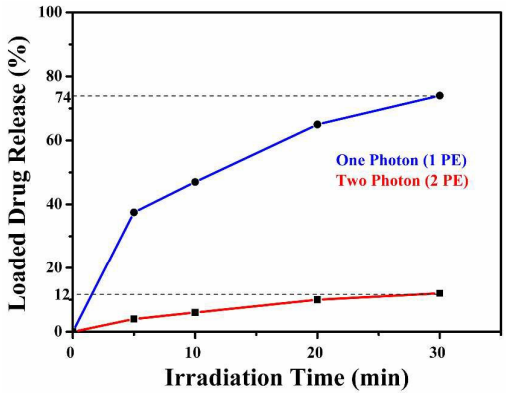


Fig. 6 HPLC profiles of percentage of loaded chlorambucil released from ONBCbl-SiQDs under one photon (≥ 410 nm) and two photon (800 nm) excitation.

Cellular Uptake and Intracellular Trafficking Studies of ONBCbl-SiQDs and SiQDs

To establish that ONBCbl-SiQDs can be used as a versatile photoresponsive nano DDS, real-time cellular uptake of SiQDs and ONBCbl-SiQDs were investigated by cellular imaging using HeLa cell. Cells were incubated with 20 µg/mL of SiQDs and ONBCbl-SiQDs in cell culture medium for 6 h and imaging was done by confocal microscope using appropriate filters. After incubation with SiQDs cells emitted bright blue fluorescence, **(Fig. 7ib).** This revealed that the SiQDs were readily internalized by the cells. On the other hand faint blue

fluorescence was observed from the HeLa cells after incubation with ONBCbl-SiQDs **(Fig. 7iib)** indicating that ONBCbl-SiQDs were also readily internalized by the cells.

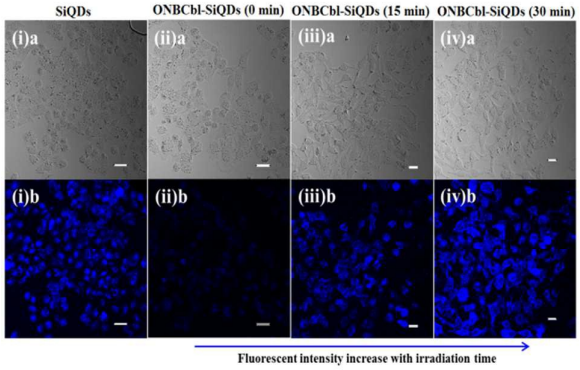


Fig. 7 Confocal bright field and fluorescence images of HeLa cells incubated with ONBCbl-SiQDs (20 μg/mL) under different irradiation times (0-30 min, time interval = 15 min): (ia-iva) bright field and (ib-ivb) corresponding fluorescence images. Scale bar = 20 μm.

Real-Time Monitoring of Drug Release

To demonstrate the real time monitoring ability of the ONBCbl-SiQDs, *in vitro* time dependent photocontrolled drug release studies on Hela cells were performed. **Fig. 7ia-b** clearly shows that before irradiation the ONBCbl-SiQDs in the HeLa cells were faintly fluorescent but upon gradual irradiation fluorescence intensity increased and finally showed characteristic fluorescence of free SiQDs **(Fig. 7iva-b)** after 30 min of irradiation, indicating photolysis of ONBCbl-SiQDs and thereby release of the anticancer drug chlorambucil.

Anticancer Efficacy of ONBCbl-SiQDs before and after Photolysis

After successful demonstration of the pronounced internalization and accumulation of SiQDs and ONBCbl-SiQDs in the HeLa cells, the *in vitro* cytotoxicity of SiQDs, chlorambucil and ONBCbl-SiQDs were evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay on the HeLa cell line. Cytotoxicity was expressed as the percentage of cell viability relative to the untreated control cells. The percentage of cell viability was plotted versus concentration of SiQDs, chlorambucil and ONBCbl-SiQDs. It was observed that cell viability remained above 80 % at different concentrations of SiQDs and ONBCbl-SiQDs in absence of light **(Fig. 8a)**, whereas an increase in cytotoxicity was observed with increase in the amount of chlorambucil **(Fig. 8a)**. Photoinduced release of chlorambucil by ONBCbl-SiQDs within the HeLa cell line and its subsequent affect on the cell viability was investigated. For that, cells were incubated with different concentrations of ONBCbl-SiQDs for 48 h and were irradiated with visible light (≥ 410 nm) for 30 min resulting in the release of the anticancer drug chlorambucil to induce cytotoxicity to HeLa cells, as confirmed by the MTT assay **(Fig. 8b)**. On the other hand, no significant cell death was observed when the cells were irradiated in the presence of SiQDs **(Fig. 8b)**, clearly indicating that the cytotoxicity caused was only because of chlorambucil which was released upon photoirradiation from ONBCbl-SiQDs. ONBCbl-SiQDs showed much lower cytotoxicity in dark compared to chlorambucil **(Fig. 8a)**. But upon irradiation ONBCbl-SiQDs showed enhanced cytotoxicity to the cancer cells in comparison to free chlorambucil **(Fig. 8b)**, because of the efficient photorelease of chlorambucil inside the cell. Thus, ONBCbl-SiQDs could be considered as an efficient biocompatible photoresponsive nano DDS for photoinduced release of anticancer drug *in vitro* for a sustained period of time.

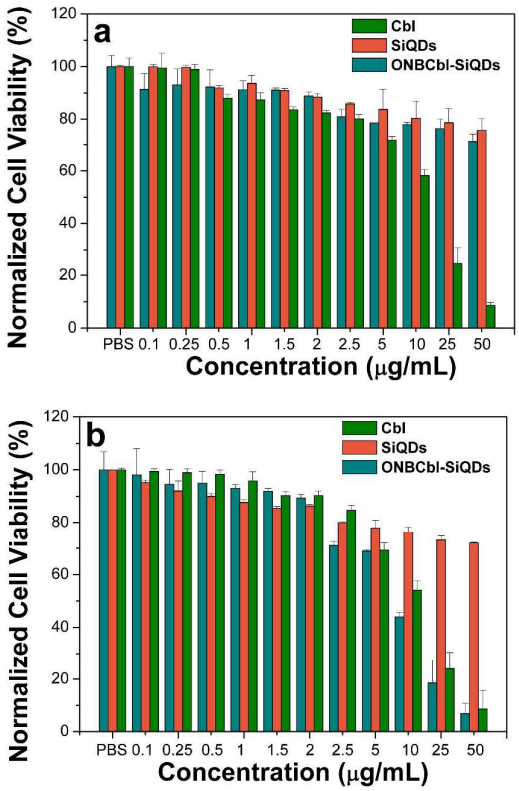


Fig. 8 (a and b) Cell viability of HeLa cells incubated with SiQDs, ONBCbl-SiQDs, and chlorambucil (Cbl), (a) before irradiation and, (b) after 30 min of irradiation. Values are presented as mean ±SD.

Conclusions

We have developed a new photoresponsive real time monitoring nano drug delivery system based on fluorescent SiQDs (ONBCbl-SiQDs) for regulated delivery of anticancer drug. We presented a new synthetic approach for synthesizing ONBCbl-SiQDs. ONBCbl-SiQDs exhibited photoinduced flourescence "off"-"on" phenomenon upon drug uncaging which was explored for real time monitoring of the drug release. Photoregulated drug delivery by ONBCbl-SiQDs upon one- and two-photon excitation (1PE and 2PE) was established by periodic exposure to light and dark conditions. Furthermore cellular internalisation of ONBCbl-SiQDs and photoinduced enhancement of cytotoxicity with respect to the concentration of ONBCbl-SiQDs inside the HeLa cell was demonstrated. Low

cytotoxicity and unique optical properties of SiQDs opened a new area of designing biocompatible photoresponsive nanoparticles based on quantum dots. In future we wish to design photoresponsive, multifunctional targeted drug delivery systems using SiQDs with precise control over the drug release.

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