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Mesoporous silica nanoparticles functionalized with thymidine derivative for controlled delivery

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Experimental Sections

Characterization: ^{1}H and ^{13}C NMR spectra were measured with a Bruker ARX 300 MHz sepctrometer. MS spectra were obtained with a JEOL JMS-700 mass spectrometer. IR spectra were obtained for KBr pellets, in the range 400–4000 cm^{-1}, with a Shimadzu FTIR 8400S instrument, and the MS spectrum was obtained with a JEOL JMS-700 mass spectrometer. Time-of-flight second ion mass spectrometer (TOF-SIMS) was analyzed on Model PHI 7200 equipped with Cs and Ga ion guns for positive and negative ion mass detection. Transmission electron microscopy (TEM) images were taken with a JEOL JEM-2100 F instrument operated at 150 kV. Images were recorded on 2k CCD (Gatan Inc. USC 1000). Scanning electron microscopic (SEM) images was taken on a Hitachi S-4500 instrument. The accelerating voltage of SEM was 5–15 kV and the emission current was 10 μA. All fluorescence spectra were recorded in RF-5301PC spectrophotometer.

Synthesis of Thymidine Derivative 1: Thymidine (1.0g, 4.12mmole) was dissolved in anhydrous DMF (10mL) followed by addition of triethylamine (3g, 29.6mmole) and triethoxysilylpropyl isocyanate (2.5g, 10.1mmol). The solution was stirred under reflux conditions for 2 days and cooled to room temperature. Water was then added and the mixture was extracted with ethylacetate (50mL). The organic layer was separated and dried over $MgSO_4$ and the removal of organic solvent was performed by vacuum to provide a crude yellow liquid product which was purified by column chromatography. ^{1}H NMR (300 MHz, DMSO-d_6) d= 11.3 (s, 1H), 7.47 (s, 1H), 7.4–7.0 (m, 3H), 6.2 (t, 1H, J = 6 Hz), 5.36–5.06 (m, 1H), 4.23–4.09 (m, 3H), 3.93 (q, 12H, J = 3.6 Hz), 2.96 (d, 2H, J = 3Hz), 2.50–2.15(m, 2H), 1.46 (m, 4H) 1.13 (t, 18H, J = 6.9 Hz), 0.55 (t, 4H, J =7.5 Hz); ^{13}C NMR (300 MHz, DMSO-d_6): 164.0, 156.2, 155.7, 150.9, 136.0, 110.2, 84.2, 82.5, 74.8, 64.3, 59.7, 43.4, 36.5, 23.4, 18.5, 15.5, 12.5, 7.6; IR (on a KBr plate, cm^{-1}): 3340(m), 2980(s), 2922(s), 2885(s), 1705(s), 1536(m); EI+ MS m/z (M) 736.26; Anal. Calcd for $C_{30}H_{56}N_4O_{13}Si_2$: C, 48.89; H, 7.66; N, 7.60; O, 28.22; Si, 7.62; Found: C, 47.73; H, 7.74; N, 7.42.

Preparation of Mesoporous Silica Nanoparticle: 2.5g of n-hexadecyltrimethylammonium bromide (C_{16}TMABr, 0.007 mol) was dissolved in 50mL of deionized water and 13.2 mL of aqueous ammonia (32wt%, 0.25mol) and 60.0mL of absolute ethanol (EtOH, 1.3 mol) to which 4.7mL of the surfactant silica precursor, tetraethylorthosilicate (0.022 mol, freshly distilled), was added dropwise under 15 minutes of 250 rpm stirring conditions to a final molar ratio of 1:0.3:11:144:58 for TEOS : C_{16}TMABr : NH_3 : H_2O : EtOH. The mixture was then stirred for 2 hours and heated to 120 °C for 20minute.

The mixture was then covered and moved into an isothermal oven set at 80 °C, where it was kept without stirring for 3 days. Spontaneous growth of suspended flocculates and precipitated particles was observed. The white precipitate was filtered and washed with distilled water followed by methanol three times and dried at 100 °C in air. To remove cationic surfactants, dried fiber-like flocculates and particles were calcined in a box furnace in air at 500 °C for 5 h, with a ramp rate of 1 °C/min.

Immoblization of Thymidine Derivative 1 onto Mesoporous Silica. T-MS was created by immobilizing the thymidine derivative **1** on the external surface of the mesoporous silica nanoparticles by first dissolving 40mg of thymidine derivative **1** in 5mL of anhydrous toluene followed by adding 40mg of MCM-41 prior to stirring and refluxing for 24 hours. The solid was collected and washed with THF to remove residual thymidine and then dried under vacuum.

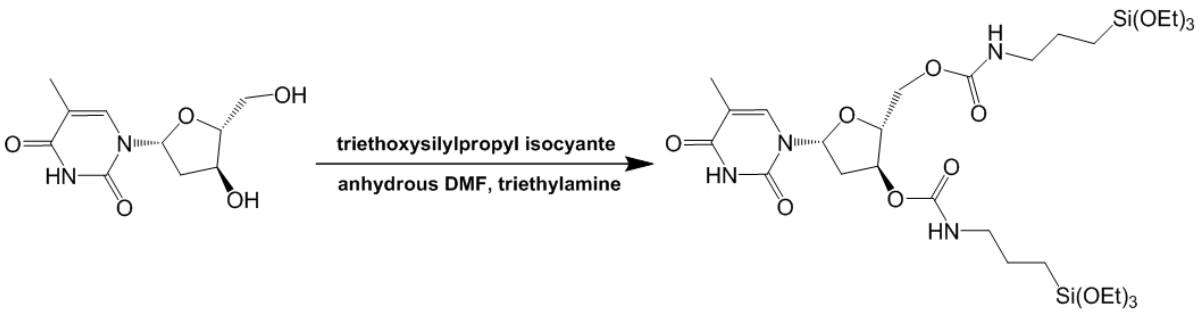
Loading of Rhodamine B to Thymidine Derivative Attached Mesoporous Silica Nanoparticle (T-MS): T-MS (100 mg) was added to rhodamine solution (2.0×10^{-3} M). The suspension of T-MS was stirred for 24 hr, then the collected solid was filtered to rinse away any surplus rhodamine. Loading was observed by the presence of an absorption peak at 566 nm by UV-Vis spectrophotometery. Because the signal of rhodamine B inside of the nanoparticles is negligible, loading was confirmed by allowing any cargo dye to diffuse out of the R-T-MS samples and into the cuvette prior to data collection.

Poly(adenine) Capping of Rhodamine-loaded T-MS (A-R-T-MS): The capping capabilities of oligo(adenine) were carried out by adding 5mg of R-T-MS to a 3.3nM solution of 5'-FAM-oligo-d$(A)_{18}$. The solution was stirred for 3 hours and then the nanoparticles were filtered to rinse away any surplus oligonucleotide. Capping was observed by the lack of a rhodamine absorption peak at 566 nm by UV-Vis spectrophotometery indicative that the rhodamine remained confined in the nanoparticles due to capping.

Poly(cytosine) Capping of Rhodamine-loaded T-MS (C-R-T-MS): The capping capabilities of oligo(cytosine) were carried out by adding 3mg of R-T-MS to a 1uM solution of 5'-FAM-oligo-d$(C)_{18}$. The solution was stirred for 3 hours and then the nanoparticles were filtered to rinse away any surplus oligonucleotide. Leaking from the capped MSN was observed by the presence of a rhodamine photoluminescence peak at ~570 nm.

Controlled Rhodamine Release Experiments by pH Induced Uncapping of C-R-T-MS and A-R-T-MS: The pH dependent release capacties of rhodamine from C-R-T-MS and A-R-T-MS (1 mg) were observed transiently by fluorescence spectrophotometric readings at 570 nm ($\lambda_{Ex= 453\ nm}$) upon exposure to ready-to-use buffer solutions at pH levels of 7, 6, 5, and 4 obtained from Sigma-Aldrich. Data was collected at 10 minutes

intervals over a 3 hour period.



Scheme S1. Synthetic route of thymidine derivative **1.**

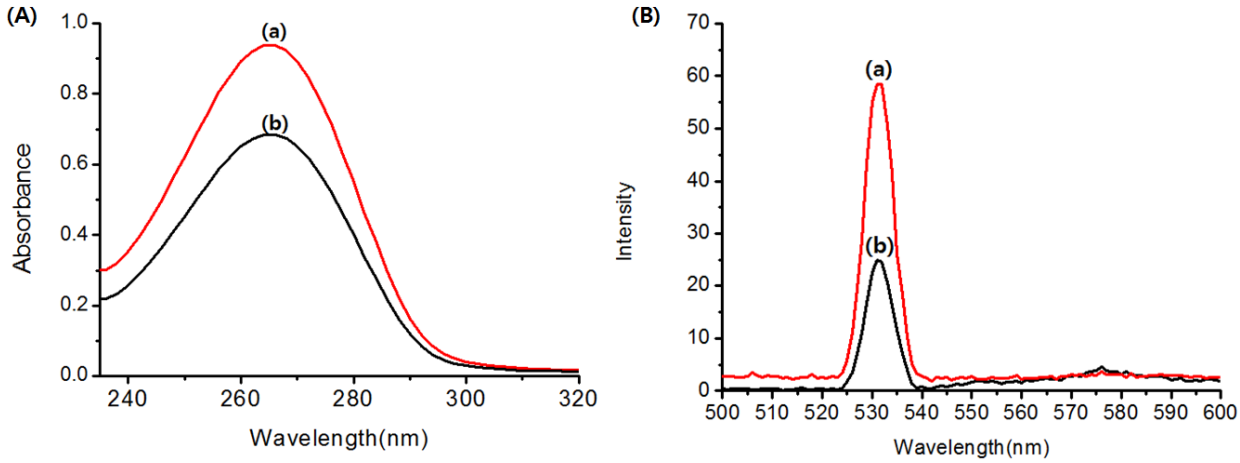


Fig. S1 (A) UV-Vis and (B) Photoluminescence spectra (a) **1** and (b) T-MSN.

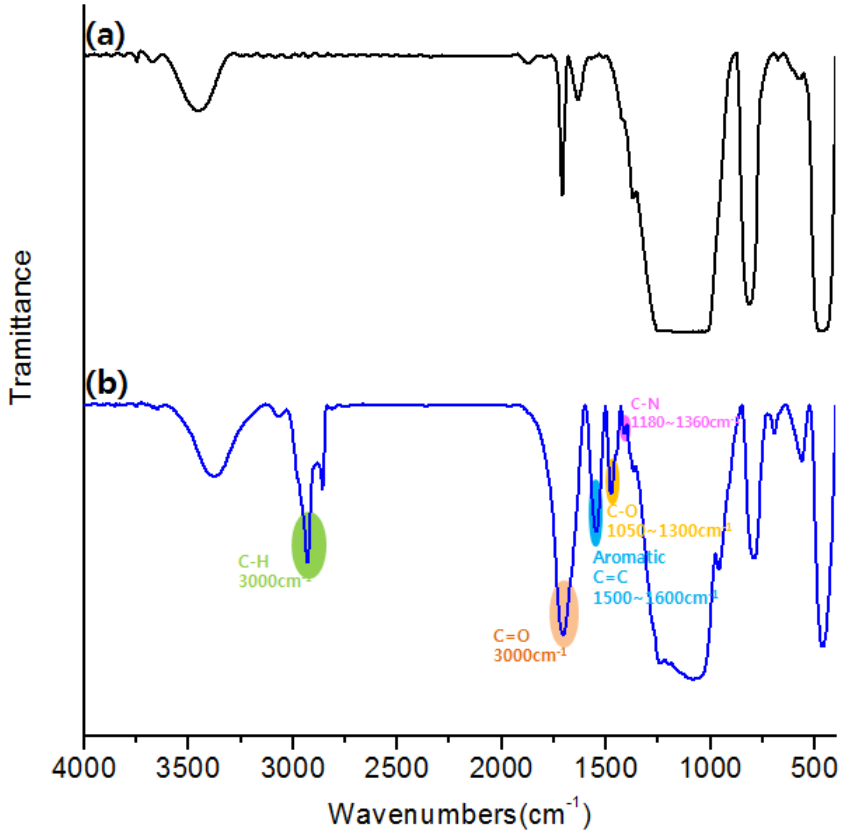


Fig. S2 IR spectra of (a) mesoporous silica and (b) thymidine derivative (**1**) attached mesoporous nanoparticle (T-MSN).

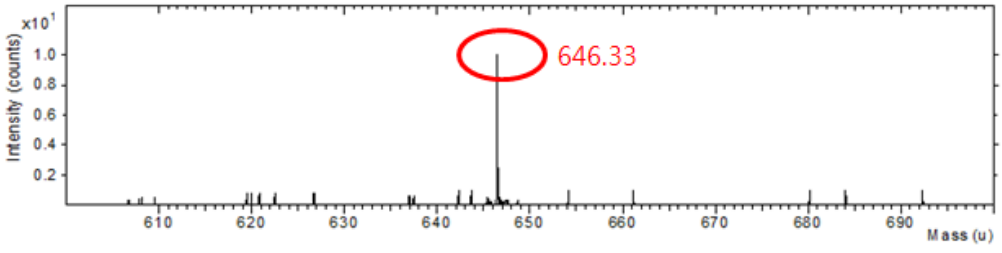


Fig. S3 TOF- SIMS spectrum of T-MSN.

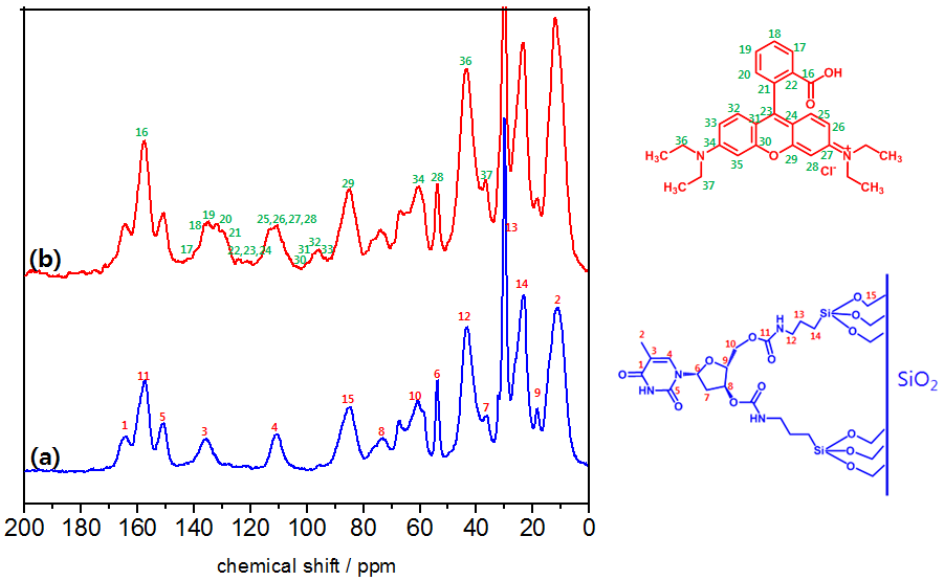


Fig. S4 ^{13}C CP-MAS spectra of (a) T-MSN and (b) rhodamine B encapsulated T-MSN (R-T-MSN).

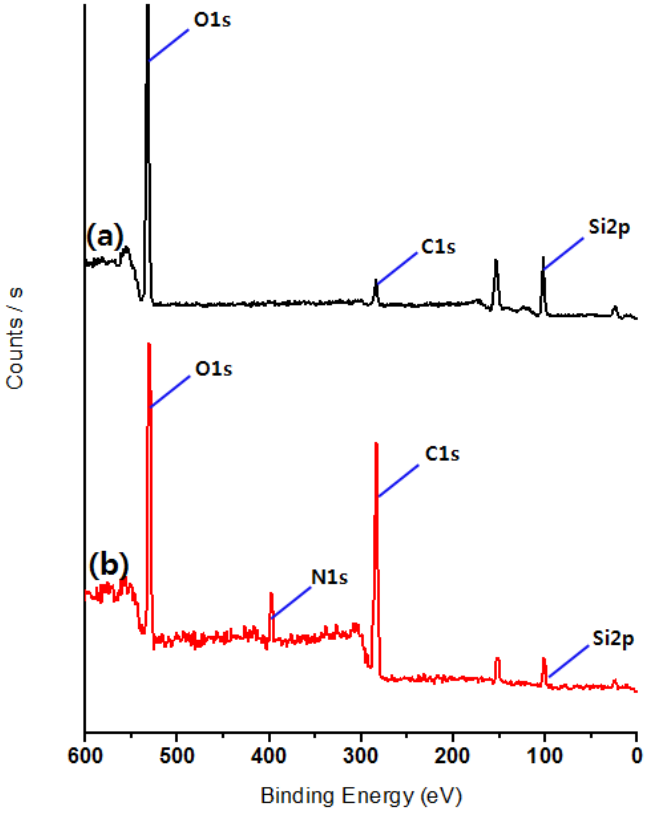


Fig. S5 XPS spectra of mesoporous silica nanoparticles (a) before and (b) after immobilization of **1** onto the surface.

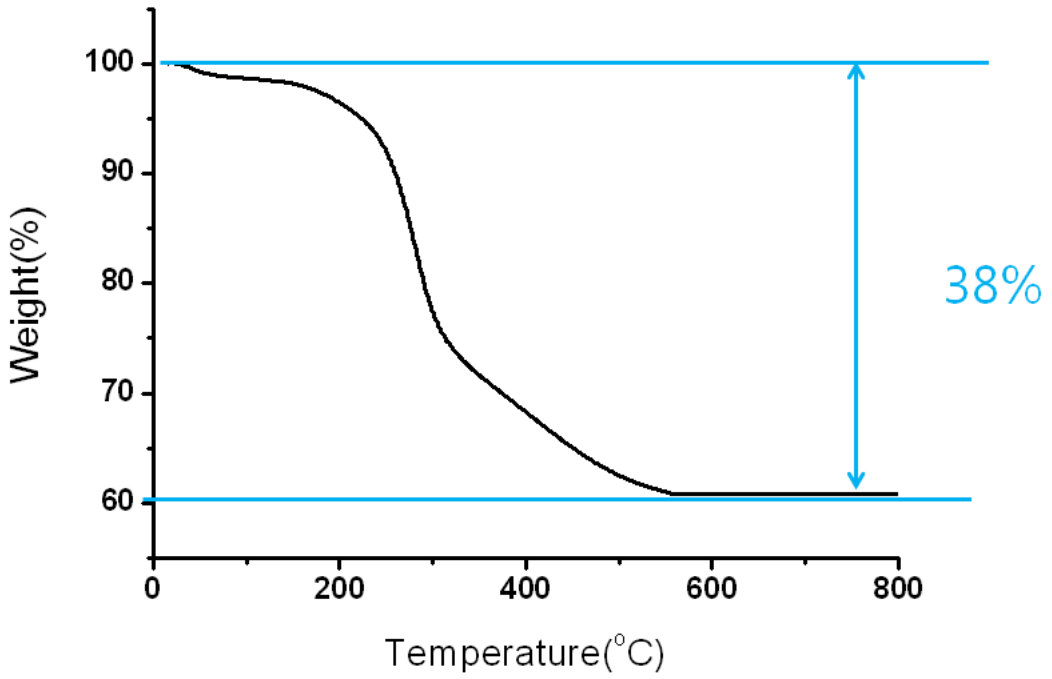


Fig. S6 TGA data of T-MSN.

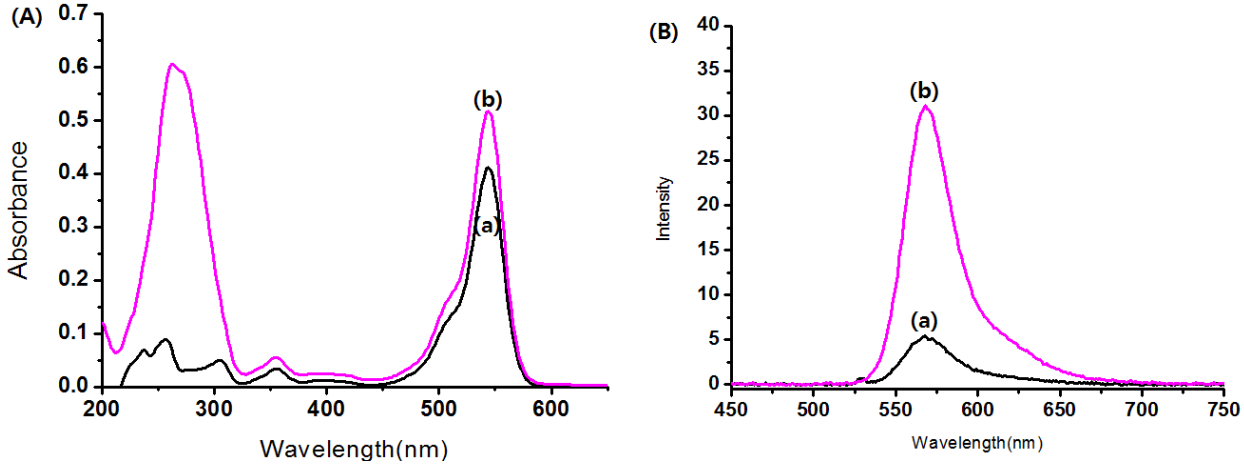


Fig. S7 (A)UV-Vis spectra of (a) suspension of rhodamine B and (b) (R-T-MSN) T-MSN after loading with rhodamine B, filtering, and re-suspension showing a large absorbance at ca. 550nm. (B) Photoluminescence spectra of (a) suspension of rhodamine B and (b) (R-T-MSN) T-MSN after loading with rhodamine B, filtering, and re-suspension showing a large peak at ca. 570nm and a smaller peak at ca. 530nm due to T-MSN. Cargo loading was effectively determined by the rhodamine peaks arising due to release from uncapped T-MSN after filtration and re-suspension.

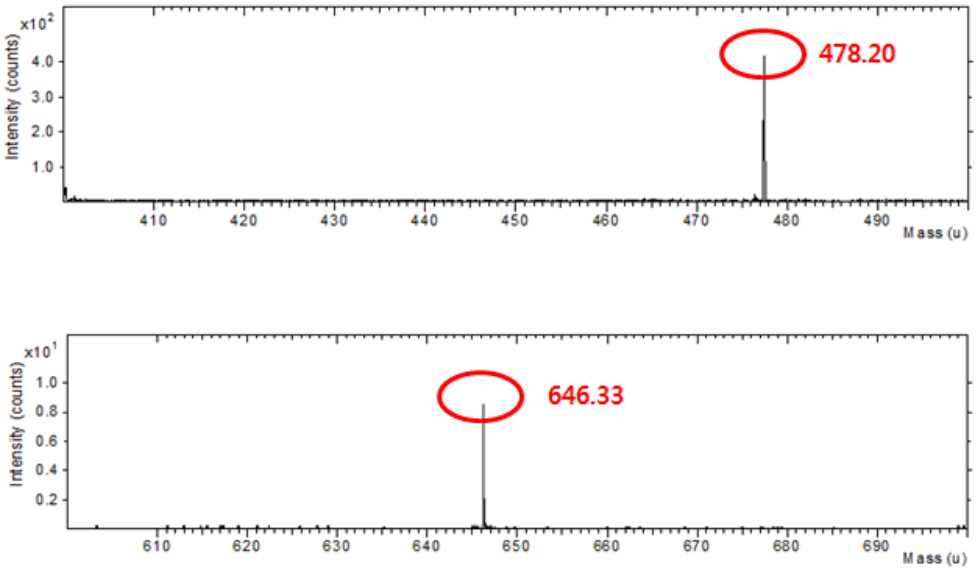


Fig. S8 TOF- SIMS spectrum of R-T-MS (top) corresponding to the presence of rhodamine B (top) and thymidine derivative **1** (bottom).

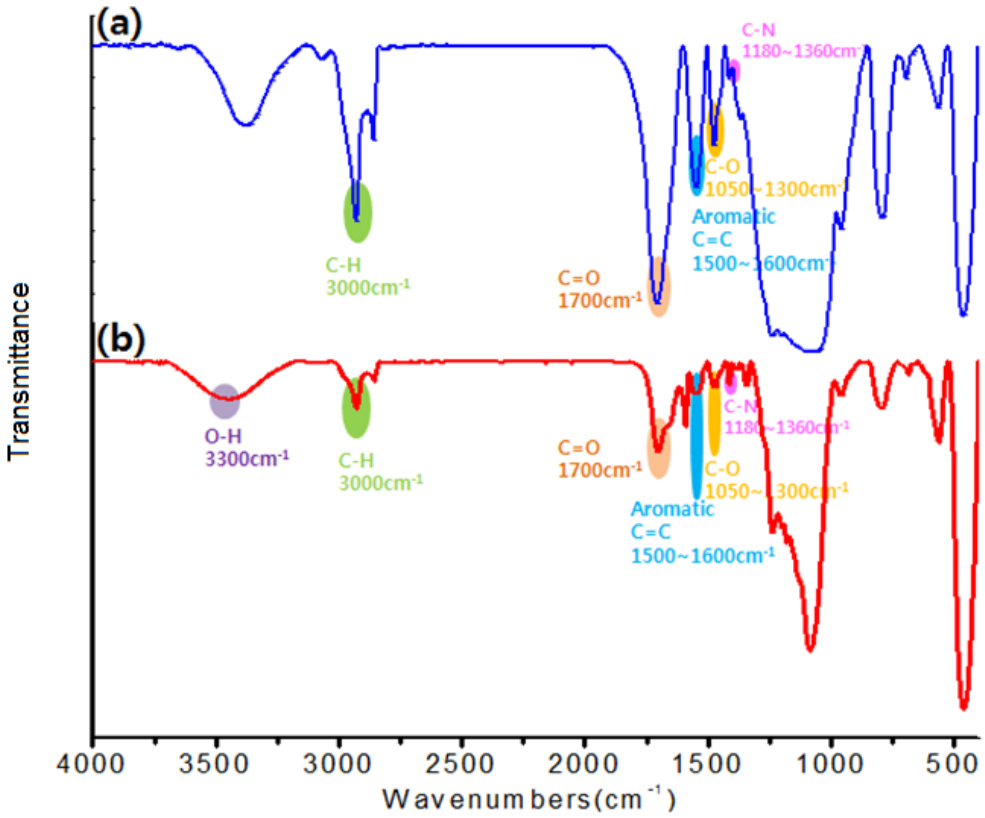


Fig. S9 IR spectra of (a) T-MSN B and (b) rhodamine B loaded T-MSN(R-T-MSN).

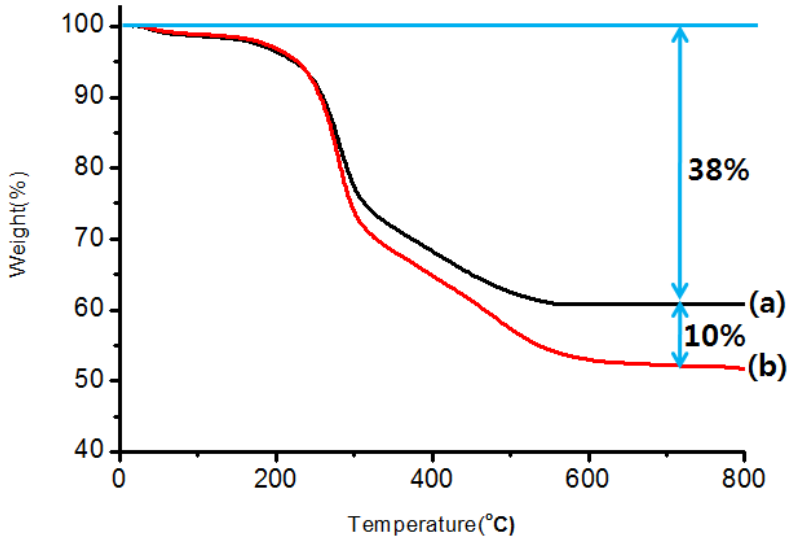


Fig. S10 TGA data of (a) T-MSN and (b) rhodamine B loaded T-MSN (R-T-MSN).

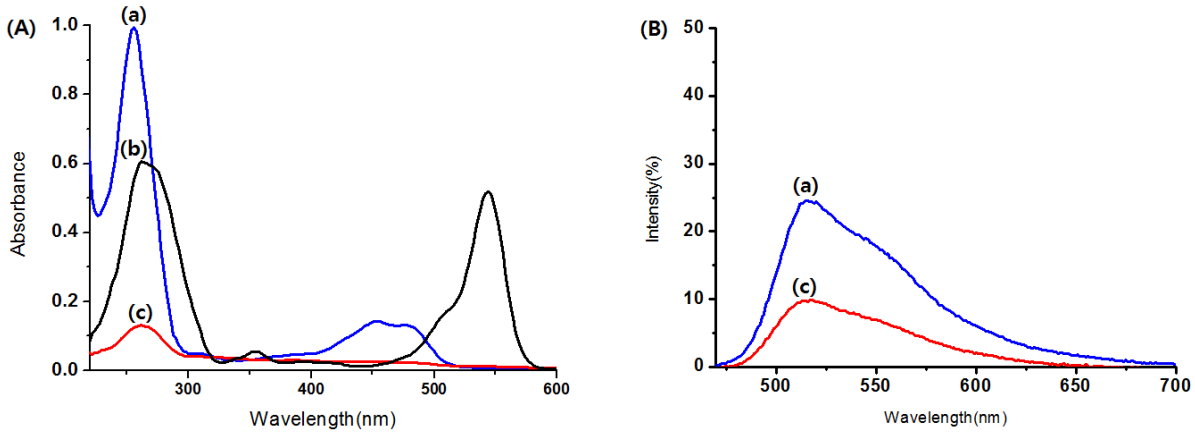


Fig. S11 (A)UV-Vis and (B) photoluminescence spectra of (a) unloaded Poly A capped T-MSN, (b) (R-T-MSN) T-MSN after loading with rhodamine B, filtered and re-suspended, and (c) (A-R-T-MSN) T-MSN after loading with rhodamine and capping with poly A to prevent leakage, filtration, and re-suspension. The UV-Vis spectra reveal that the rhodamine can leak from the un-capped solution (b) by the observed peak at ca. 550nm. From the photoluminescence spectra we can see that poly A capping of rhodamine loaded T-MSNs effectively prevents leakage of rhodamine into the re-suspended solution as is observed by (c) the lack of a rhodamine peak at ca. 570nm. Please note that the relative height of the photoluminescence peaks of (a) and (b) is not significant.

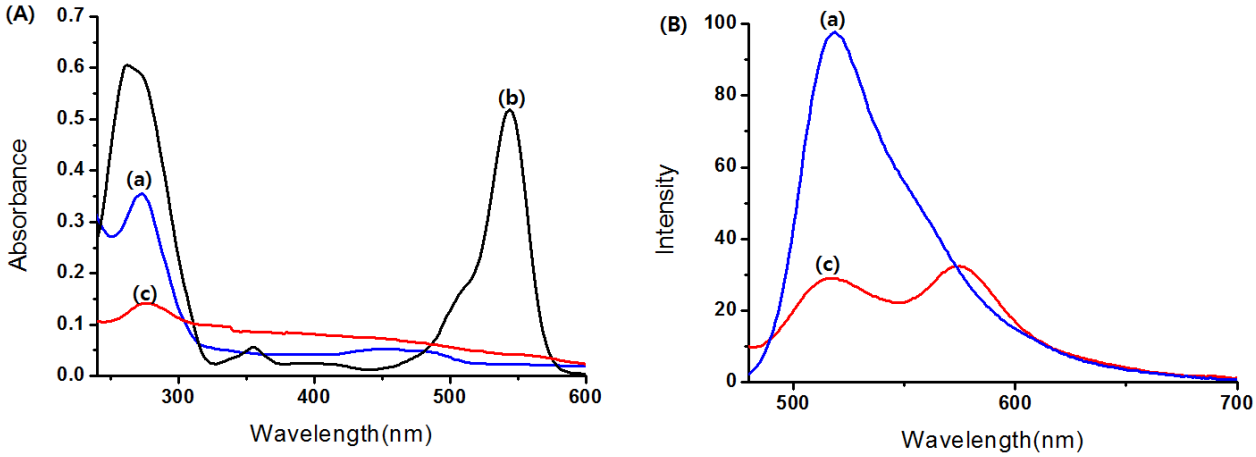


Fig. S12 (A)UV-Vis followed by (B) photoluminescence spectra of (a) unloaded Poly C capped T-MSN, (b) (R-T-MSN) T-MSN after loading with rhodamine B, filtered and re-suspended, and (c) (C-R-T-MSN) T-MSN after loading with rhodamine and capping with poly C, filtration, and re-suspension. The UV-Vis spectra reveal that the rhodamine can leak from the un-capped solution (b) by the observed peak at ca. 550nm. From photoluminescence data, the poly C capping of rhodamine loaded T-MSN (c) resulted in noticeable leakage of rhodamine into the re-suspended solution as is observed by the ca. 570nm rhodamine peak. Please note that the relative height of the photoluminescence peaks of (a) and (b) is not significant. In addition, the three hour time taken between UV-Vis experiments and photoluminescence experiments allowed for increased observation of the slow release of rhodamine from poly C capped MSNs by photoluminescence (c).

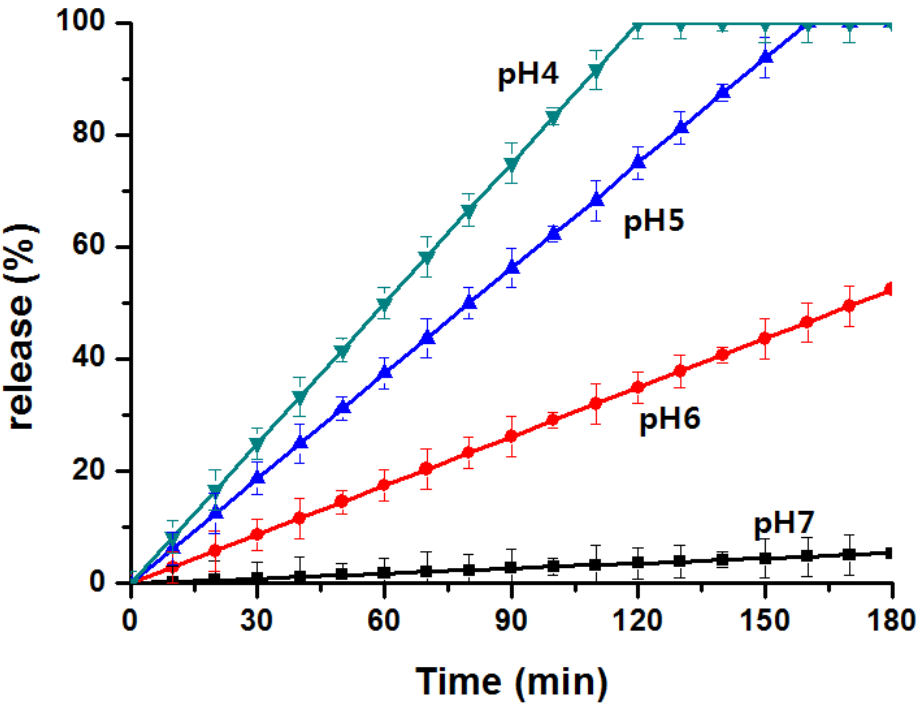


Fig. S13 pH dependent release profile of rhodamine B using poly C capped C-R-T-MSN. A noticeable leakage of approximately 4% of loaded rhodamine was observed over a period of 3 hours at pH 7 indicating insufficient encapsulation by poly C capping.