Electronic Supplementary Material (ESI) for Nanoscale.
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NIR upconversion fluorescence glucose sensing and glucose-responsive insulin release of carbon dot-immobilized hybrid microgels at physiologcial pH

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Materials and methods

Materials: D(t)-Glucose was purchased from ACROS, and all other chemicals were purchased from Aldrich. The lyophilized fluorescein isothiocyanate-labeled insulin (FITC-insulin) from bovine pancreas (～5800 Da), 4-vinylphenylboronic acid (VPBA), acrylamide (AAm), N,N′-methylenebisacrylamide (BIS), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), sodium dodecyl sulfate (SDS), HCl (37%), were used as received without further purification. The water used in all experiments was of Millipore Milli-Q grade.

Synthesis of the carbon dots (CDs): CDs were synthesized via an acid-assisted ultrasonic and thermal treatment of glucose. In a typical synthesis, glucose (2.70 g) was dissolved in deionized water (10 mL). After intense sonication for 20 min, 30.0 mL of HCl (37 wt %) was slowly added into the above solution. The mixed solution was then treated ultrasonically for 8 h and transferred into a 50 mL Teflon-lined stainless autoclave. The precursor solution was heated to and maintained at 200 °C. After 24 h, the solution was cooled naturally to room temperature. The resulted CDs were purified with repeated centrifugation and redispersion in water for three cycles so that larger CDs could be removed from products. Finally, the aqueous dispersion of CDs was dialyzed for 7 days (Spectra/Pormolecularporous membrane tubing, cutoff 12 000–14 000) at room temperature (≈22 °C). The dialytic aqueous dispersion of CDs was then collected and dried to get solid CDs.

Synthesis of poly(VPBA-AAm)-CDs hybrid microgels: The CDs with strong NIR upconversion fluorescence were synthesized and collected using the previously reported method. In a 250 mL round-

bottom flask equipped with a stirrer, a N_2 gas inlet, and a condenser, AAm (0.102 g) was added into aqueous solution (97 mL, 0.1 mg/mL) of CDs, and then the mixture solution was heated to 70 ^{o} C for 30 min. After that, VPBA (1.84 g), BIS (0.0736 g) and SDS (0.0508 g) was added into above mixture solution under stirring. After the temperature was maintained for 60 min under N_2 purge, the polymerization was initiated by adding 3.00 mL AAPH solution of 0.105 M. The polymerization reaction was allowed to proceed for 6 h. The solution was centrifuged at 16,000 rpm (30 min, Thermo Electron Co. SORVALL®RC-6 PLUS superspeed centrifuge) with the supernatant discarded and the precipitate redispersed in 100 mL deionized water. This procedure was repeated for three times. To remove unreacted monomers and free CDs, the resultant hybrid microgels with a volume of 100 mL was further purified by 10 days of dialysis (Spectra/Por®molecularporous membrane tubing, cutoff 12000-14000 Dalton MWCO) against very frequently changed water at room temperature.

Preparation of insulin-loaded poly(VPBA-AAm)-CDs hybrid microgels: FITC-insulin was loaded into the hybrid microgels by complexation method. A stock solution of FITC-insulin (1 mg/mL) was prepared in 0.005 M phosphate buffer saline (PBS) of pH = 7.40 and stored in the refrigerator (4 °C). The hybrid microgels (5 mL, 0.5 mg/mL) dispersion placed in a vial was stirred in an ice water bath for 30 min and 1 mL of FITC-insulin solution was then added dropwise to the vial. The immediate clouding revealed the hydrogen bonding complexation of the hydroxyl groups in the insulin molecules with the hydroxyl/amide groups on the poly(VPBA-AAm) microgel network chains. After stirring overnight, the suspension was centrifuged at 16,000 rpm for 30 min at 22 °C. To remove free insulin, the precipitate was redispersed in 5 mL of PBS of pH = 7.40 and further purified by repeated centrifugation and washing. Finally, the precipitate was redispersed in 5 mL of PBS of pH = 7.40. All the upper clear solutions were collected and diluted to a fixed volume. The concentration of the remained free insulin was determined by fluorescence spectrometry at 518 nm upon excitation at 492 nm. The amount of loaded FITC-insulin in hybrid microgels was calculated from the difference of the initial amount of insulin used for loading process and the remained free insulin in the upper solutions. The loading content is expressed as the mass of loaded drug per unit weight of dried hybrid microgels.

Insulin release from poly(VPBA-AAm)-CDs hybrid microgels: The in vitro release of FITC-insulin from the hybrid microgels was evaluated by the dialysis method. The FITC-insulin loaded hybrid microgels were redispersed in 5 mL PBS solution (0.005 M, pH=7.4). Four dialysis bags filled with 1 mL such FITC-insulin loaded hybrid microgel dispersion were immersed in 50 mL 0.005 M buffer solutions

of pH = 7.40 but at different glucose concentrations (0 mM, 5 mM, 10 mM and 20 mM) at physiological temperature of 37 ^{o}C. The released insulin outside of the dialysis bag was sampled at defined time period and assayed by fluorescence spectrometry. Cumulative release is expressed as the total percentage of insulin released through the dialysis membrane over time.

In vitro cytotoxicity of free CDs, poly(VPBA-AAm) microgels and poly(VPBA-AAm)-CDs hybrid microgels: In this study, B16F10 cells and 4T1 cells were respectively cultured in the 96 wells microplate with 100 μL medium containing about 2,000 seeded cells in each well. After an overnight incubation for attachment, the medium was removed and another 100 μL medium containing different amount of free CDs, poly(VPBA-AAm) microgels and poly(VPBA-AAm)-CDs hybrid microgels was added to make the final exact concentration of 100, 50, 25, and 12.5 μg/mL for each sample, respectively. Wells with the normal medium only were used as control. After incubated for 24 h, 10 μL of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/ml in PBS) was added into the wells. The wells were further incubated in a humidified environment of 5 % CO_2 and 37 °C for 2 h. The medium were removed after 2 h and 100 μL of DMSO solution was added. The plates were gently agitated until the formazan precipitate was dissolved, followed by measurement of optical density value by spectrophotometer at 570 nm and 690 nm.

Histopathological evaluation:

Principles of animal care and experimentation to be followed are those found in the Guide for the Care and Use of Laboratory Animals (DHEW Pub. No. (NIH) 85-23 revised 1996). College of Staten Island operates in accordance with an approved Animal Welfare Assurance on file with the Office for Protection from Research Risks. Animal Colony is under the supervision of veterinarians and the City University of New York Animal Care and Use Committee (ACUC), all of whom assure compliance with NIH and USDA guidelines.

After an intracardial perfusion of buffered 10% formalin, whole organs (heart, kidney, liver, lung and spleen) of C57BL/6 mice were removed through necropsy and post fixed in the same fixative for 48 h and embedded in paraffin processed for histology, sliced into 5 μm sections, and stained with hematoxylin and eosin (H&E) according to standard clinical pathology protocols. A veterinary pathologist was then consulted to evaluate if any signs of acute toxicity were present in these clearance organs. Samples were submitted pathology assay 5 days after intravenous administration of the hybrid nanogels (n = 3) at a concentration of 1 mg/ mL and compared to mice receiving no injection (n=3).

Characterization: Transmission electron microscopy (TEM) images were taken on a FEI TECNAI transmission electron microscope at an accelerating voltage of 100 kV. Field emission scanning electron microscopy (FE-SEM) imaging was performed on an AMRAY 1910 Field Emission Scanning Electron Microscope. Two-photon imaging was performed using an Olympus FV1000 MPE BX61 multi-photon microscope with excitation wavelength at 900 nm. The UV-vis absorption spectra were obtained on a Thermo Electron Co. Helios β UV-vis Spectrometer. The FT-IR spectra were recorded with a Nicolet Instrument Co. MAGNA-IR 750 Fourier transform infrared spectrometer. The UV light was provided by a 250 W high-pressure fluorescent Hg lamp. The PL spectra were obtained on a JOBIN YVON Co. FluoroMax®-3 Spectrofluorometer equipped with a Hamamatsu R928P photomultiplier tube, calibrated photodiode for excitation reference correction from 200 to 980 nm, and an integration time of 1 s. Dynamic light scattering (DLS) was performed on a standard laser light scattering spectrometer (BI-200SM) equipped with a BI-9000 AT digital time correlator (Brookhaven Instruments Inc.) to measure the hydrodynamic radius (R_h) distributions. A Nd:YAG laser (150 mW, 532 nm) was used as the light source. The hybrid microgel dispersion was passed through Millipore Millex-HV filters with a pore size of 0.80 μm to remove dust before the DLS measurement.

Supplementary figures

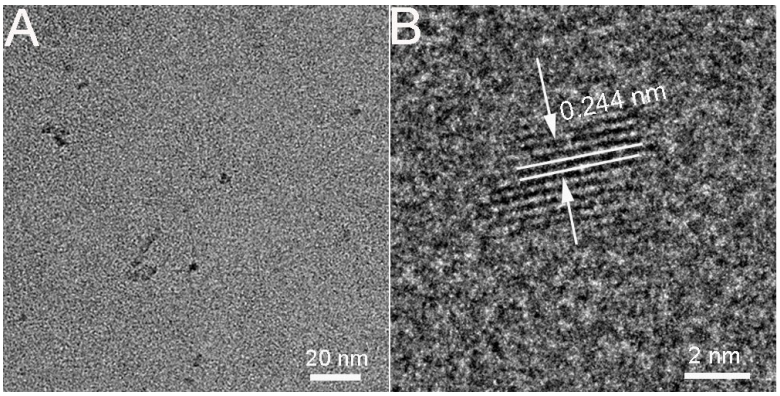

Figure S1. (A) TEM image and (B) lattice fringe of the as-obtained CDs, respectively.

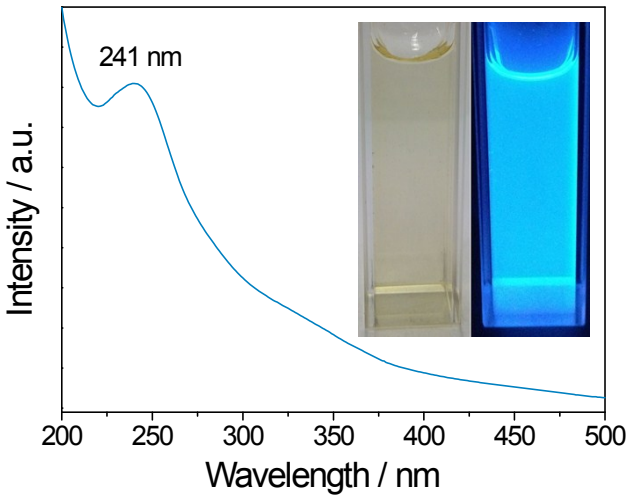

Figure S2. Typical UV-Vis spectrum of the as-obtained CDs. The inset is photographs of the aqueous dispersions of the CDs irradiated without (left) and with (right) a UV light (wavelength = 365 nm).

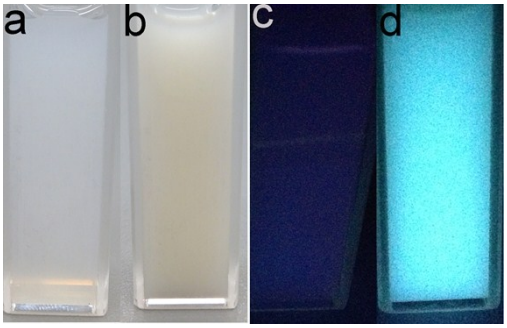

Figure S3. The photographs of the aqueous dispersions of poly(VPBA-AAm) microgels (a and c) and poly(VPBA-AAm)-CDs hybrid microgels (b and d) without/with an exposure to the UV light (365 nm).

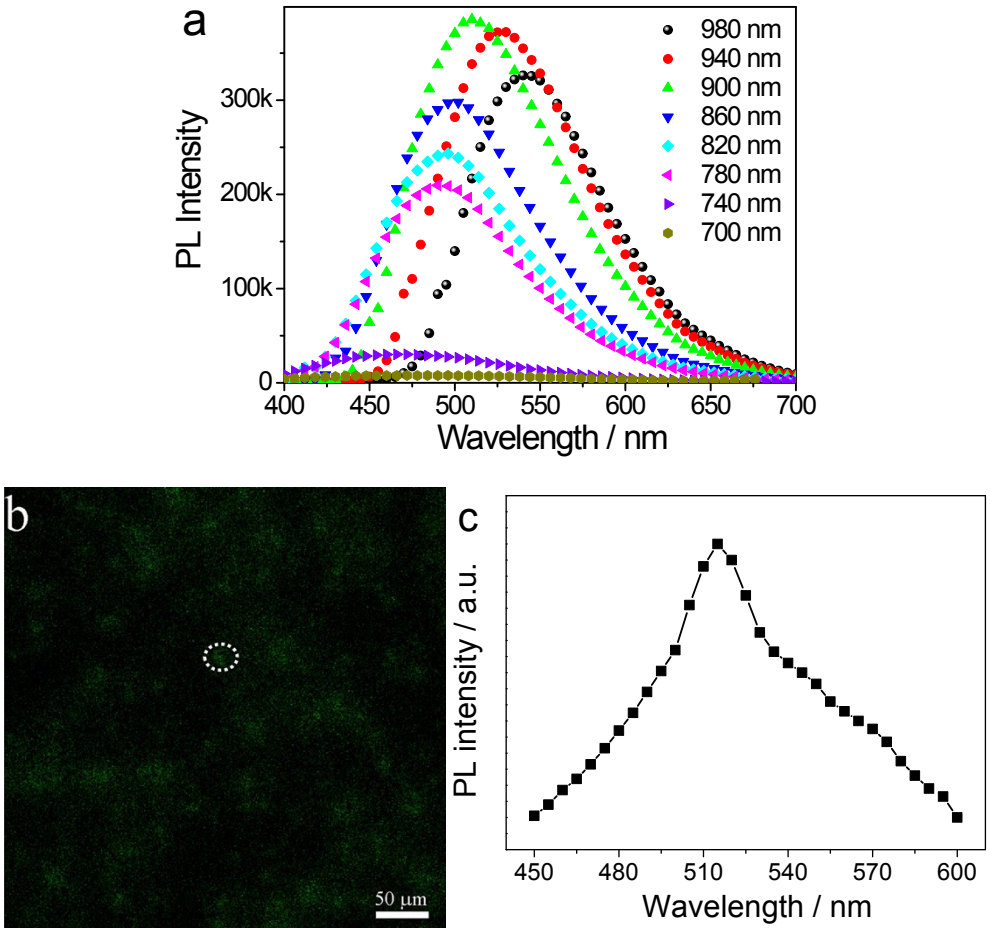

Figure S4. (a) Upconverted PL spectra of the free CDs in water obtained from FluoroMax®-3 Spectrofluorometer under excitation wavelengths from 980 nm to 700 nm. (b) Two-photon fluorescence image and (c) local PL spectrum from the white line circled region in (b) of the free CDs obtained from a two-photon fluorescence microscope under the excitation of a femtosecond infrared laser with a wavelength of 900 nm. As shown in Figure S4b, the free CDs display bright green fluorescence under the excitation of the femtosecond infrared laser of 900 nm. The local PL spectrum of the free CDs in Figure S4c obtained from the white-line ringed region in Figure S4b exhibit a maximum emission wavelength

around 512 nm, indicating that the free CDs display upconverted PL under the excitation of femtosecond laser of 900 nm. The maximum emission around 512 nm of this local PL spectrum in Figure S4c excited by the femtosecond laser of 900 nm is similar to the maximum emission around 510 nm of the PL spectrum in Figure S4a excited by xenon lamp at 900 nm obtained from the Spectrofluorometer, confirming the real upconverted PL of the free CDs.

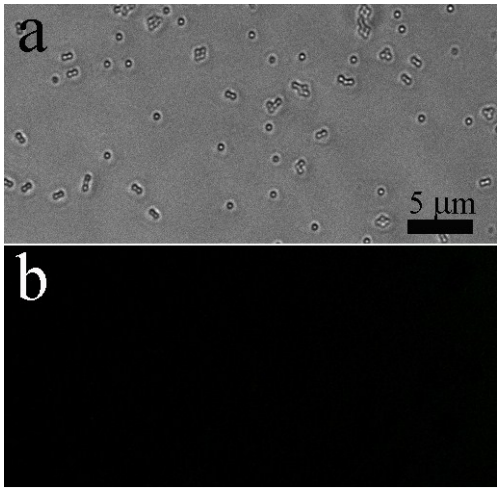

Figure S5. Transmission (a) and two-photon fluorescence (b) images of poly(VPBA-AAm) microgels. Excitation laser wavelength is 900 nm.

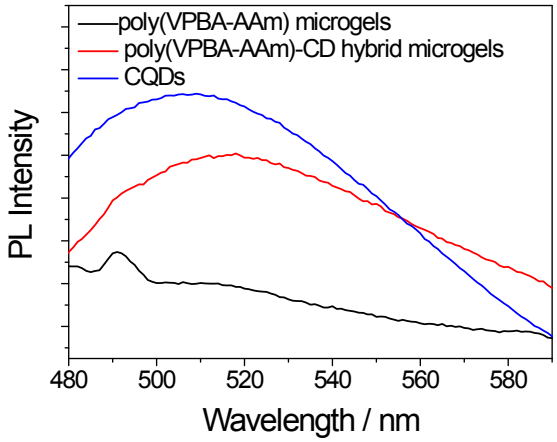

Figure S6. Typical PL spectra of the free CDs, poly(VPBA-AAm) microgels, and poly(VPBA-AAm)-CDs hybrid microgels, respectively. Excitation wavelength = 900 nm.

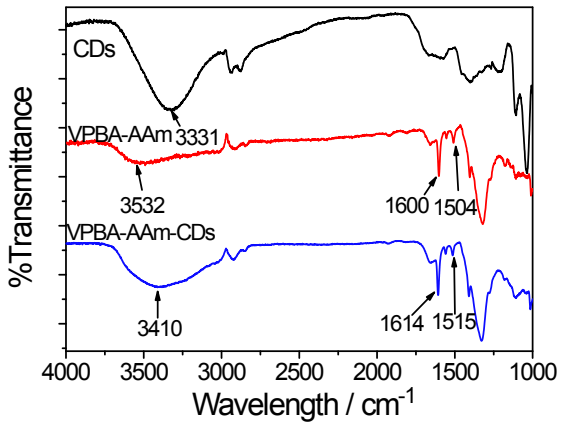

Figure S7. FT-IR spectra of the free CDs, poly(VPBA-AAm) microgels, and poly(VPBA-AAm)-CDs hybrid microgels, respectively.

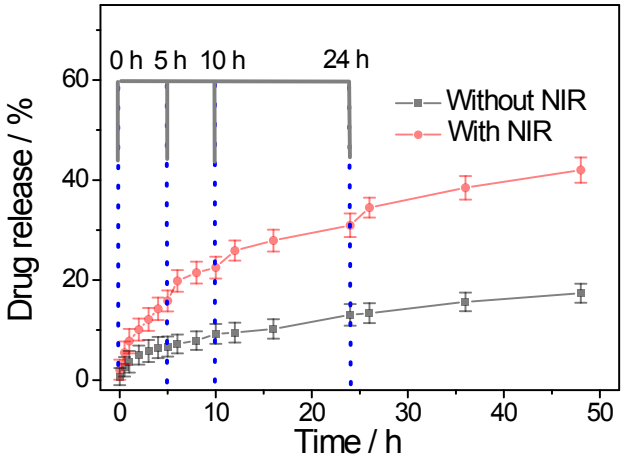

Figure S8. Release profiles of insulin from the hybrid microgels in PBS of pH = 7.4 with and without an exposure to the 1.5 W/cm^2 NIR light for 5 min at the releasing stages of 0, 5, 10, and 24 h, respectively, at a constant temperature of 37 ^{o}C.

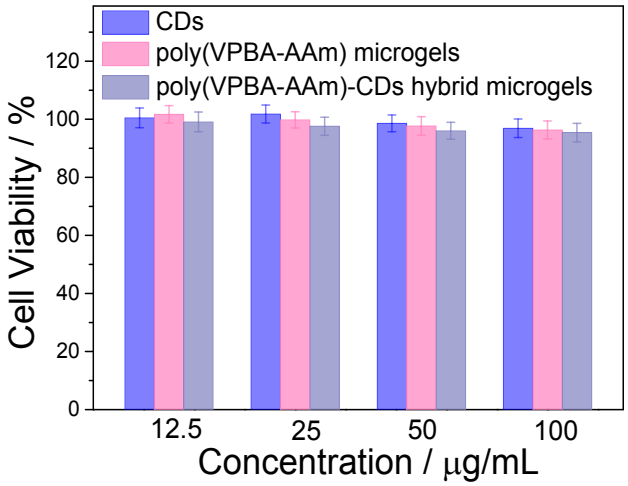

Figure S9. In vitro cytotoxicity of CDs, poly(VPBA-AAm) microgels, and poly(VPBA-AAm)-CDs hybrid microgels on HEK293T cells at different concentrations performed by assessing the cell viability for 72 h.