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Multifaceted thermoresponsive poly(N-vinylcaprolactam) coupled with carbon dots for biomedical applications

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ABSTRACT

A fluorescent thermoresponsive polymer consisting of poly(N-vinylcaprolactam) (PVCL) coupled with carbon dots (CDs) (PVCL–CDs) was synthesized by reacting a carboxyl-terminated PVCL derivative with CDs via N-hydroxysuccinimide and N-(3-(dimethylamino)propyl)-N-ethylcarbodiimide hydrochloride coupling. The temperature-dependent fluorescence properties of this material were studied for biomedical applications. Fluorescence quenching in PVCL–CDs was observed above the lower critical solution temperature (LCST) due to thermo-induced aggregation of the PVCL chains. This fluorescent thermoresponsive PVCL–CDs showed good biocompatibility and was demonstrated as a thermometer for sensing intracellular temperatures and also as a marker for bioimaging. In addition, PVCL–CDs showed a significant fluorescence turn-on response to proteins above the LCST, which allows for the utilization of this material in biosensors. Thus, PVCL–CDs, with its tuneable size, low cytotoxicity, good photostability, ease of bioconjugation, and resistance to metabolic degradation, is a novel material for biomedical applications.

1. Introduction

Multifunctional nanomaterials are becoming increasingly important for applications in a wide range of fields including imaging, biosensing, diagnostics, drug delivery, catalysis, photonics, and medicine [1]. For diagnostic and therapeutic purposes, fluorescent nanomaterial with durable and considerable brightness, sufficient water dispersibility, good biocompatibility, and ease of bioconjugation is of practical interest [2]. Among many fluorescent materials, semiconductor quantum dots (QDs) are a major milestone due to the tuneable and narrow features of their emission spectrum, bright fluorescence, high photostability, and resistance to metabolic degradation in biomedical applications. However, QDs comprise potentially toxic elements such as cadmium and selenium, which may be released and are lethal to cells and living organisms. In addition, QDs often agglomerate in biological systems due to their highly dynamic surface structures [3,4]. These intrinsic restraints of QDs markedly hamper their physiological utility in clinical implementations, thus initiating the unremitting search for less toxic fluorescent nanomaterials with improved biochemical properties.

In this regard, carbon dots (CDs) have been studied as a new class of fluorescent and discrete quasi-spherical carbon nanomaterials with sizes less than 10 nm and fascinating merits like simple fabrication processes, low production costs, chemical inertness, lack of optical blinking, low photobleaching, low cytotoxicity, and excellent biocompatibility [5–8]. The unique photophysical and chemical properties of CDs have advanced the development of novel imaging probes, high performance nanosensors, and multifunctional nanocomposites for addressing challenges in biological research. The CDs can be grafted onto synthetic biocompatible polymers to generate biologically compatible composites with good stability and low toxicity. Most synthetic polymers treated with the CDs are transparent in the visible region of the electromagnetic spectrum and hence do not interfere with the fluorescence of CDs [9,10]. Grafted polymers also prevent agglomeration and offer good processability. Hence, engineering the surface of CDs will allow for the utilization of CDs in appealing biological applications.

Temperature-sensitive polymers with lower critical solution temperatures (LCSTs) in aqueous solutions can be exploited to produce luminescent materials with thermoresponsive characteristics. Poly(N-vinylcaprolactam) (PVCL) is a promising polymer with an LCST transition ranging from 30 to 50 °C depending on its molecular weight [11]. PVCL has many advantages, such as solubility in water and organic solvents, non-toxicity, and biocompatibility. An important advantage of PVCL over poly(N-isopropylacrylamide) (PNIPAM), a typical thermosensitive polymer with an LCST, is that the amide group in the lactam ring attached to the backbone does not hydrolyse easily. Even when PVCL is hydrolysed to its polymeric carboxylic acid form, no toxic amine is produced as is with hydrolysed PNIPAM [12]. Thus, PVCL has been applied to many biomedical fields, such as drug delivery, thermometers, and biomaterials [13,14].

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Water-soluble nano-sized semiconductor QDs with a protective layer of covalently grafted PNIPAM (PNIPAM–QD) were synthesized by Ye et al. and the reversible association and dissociation of PNIPAM–QD upon altering the solution temperature was studied [15]. They also studied a thermoresponsive PNIPAM–QD platform based on PNIPAM brushes grafted from a gold substrate with QDs covalently attached to the PNIPAM layer [16], potentially useful as a smart and tuneable material in biological applications. However, the toxicity of the QDs and PNIPAM hampers its application in biological fields. Additionally, multi-stimuli responsive hyper-branched polyethyleneimine and isobutyric amide-functionalized CDs were studied by Yao et al. in which the thermoresponsive unit comprised amide bonds, which also yielded toxic low molecular weight amines upon hydrolysis [17]. Thus, a new system with high biocompatibility, low toxicity, stability and biological functionality is highly desirable.

Herein, we reported a new fluorescent thermoresponsive polymer which was developed by coupling PVCL with CDs (PVCL–CDs) through the reaction of a carboxyl-terminated poly(N-vinylcaprolactam) derivative (PVCL-COOH) with CDs via N-hydroxysuccinimide (NHS) and N-(3-(dimethylamino)propyl)-N-ethylcarbodiimide hydrochloride (EDC·HCl) coupling. The fluorescent thermoresponsive properties of this material were studied for applications as protein sensors, fluorescent intracellular thermometers, and bioimaging markers. The combination of thermoresponsive PVCL with fluorescent CDs produces a new fluorescent material with good biocompatibility.

2. Experimental

2.1. Materials

N-vinylcaprolactam (NVCL, Sigma Aldrich, 98%) was purified by recrystallization from hexane, dried overnight under vacuum at 25 °C, and stored at 4 °C. Ethylene diamine, citric acid, 2,2′-azobis[N-(2-carboxyethyl)-2-methylpropionamidine]hydrate (VA-057), N-hydroxysuccinimide (NHS), N-(3-(dimethylamino)propyl)-N-ethylcarbodiimide hydrochloride (EDC·HCl), high glucose Dulbecco's modified Eagle's medium (DMEM), foetal bovine serum (FBS), phosphate buffer saline (PBS), (3-(4,5-dmethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 98%), bovine serum albumin (BSA), lysozyme, and all other reagents and solvents were purchased from Sigma Aldrich and used as received.

2.2. Synthesis of carbon dots (CDs)

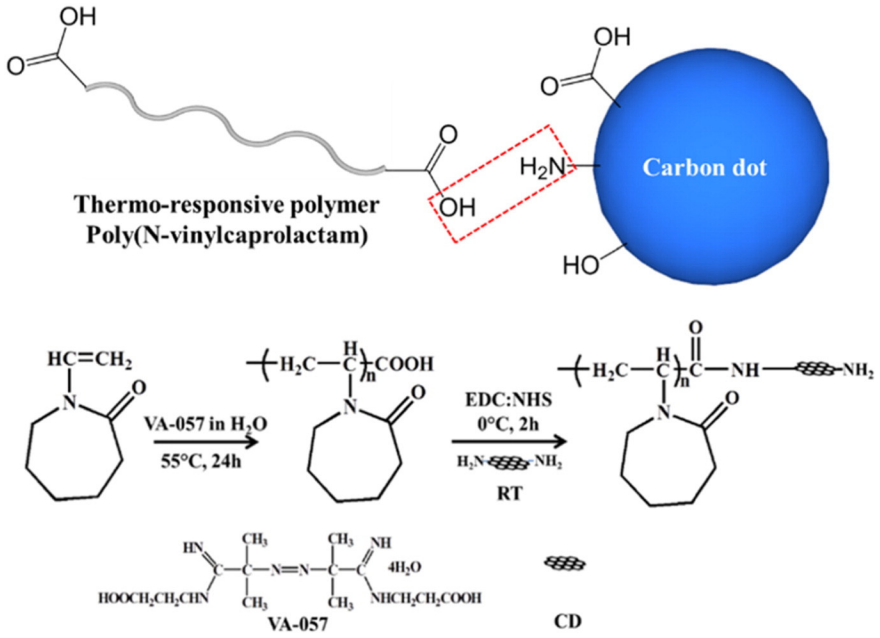
CDs were synthesized by the previously reported method [18]. Following is the brief description of the synthesis of CDs. Citric acid monohydrate (1.05 g) and ethylenediamine (0.334 mL) were dissolved in 100 mL of deionized (DI) water. The solution was added to a Teflon hydrothermal reactor (autoclave) and the temperature was maintained at 250 °C for 5 h. Then, the solution was cooled in cold water and purified by dialysis. The resultant CDs (Scheme 1) have amine functional groups on their surfaces (supporting information, Fig. S1), which can be reacted with carboxylic groups via NHS and EDC·HCl coupling.

2.3. Synthesis of carboxyl-terminated poly(N-vinylcaprolactam) (PVCL-COOH)

The synthetic scheme for PVCL-COOH is summarized in Scheme 1. Briefly, 1000 mg of VCL and 10 mg of the VA-057 initiator were dispersed in separate Schlenk flasks containing 12 and 1 mL of water, respectively, and purged with N2 for 30 min. Then, the Schlenk flask containing the initiator was immersed in an oil bath and maintained at 55 °C for 30 min. Finally, the VCL solution was injected into the Schlenk flask and the reaction proceeded at 55 °C for 24 h. The synthesized PVCL-COOH was purified by precipitation in cold ethanol [19].

2.4. Synthesis of coupling carboxyl-terminated poly(N-vinylcaprolactam) with carbon dots (PVCL–CDs)

To a 1 wt.% aqueous solution of PVCL-COOH (10 mL), 1 mL of a EDC:NHS (2:1 molar ratio) solution was added and stirred at 0 °C for 2 h. Then, 0.1 mL of the aqueous CDs solution (14.3 mg/mL) was added and stirred at room temperature overnight in the dark. The solution was then kept in an oven at 50 °C for 2 h. The PVCL-grafted CDs (PVCL–CDs) aggregated at the bottom and sides of the vial and the supernatant containing unreacted CDs was decanted. The overall reaction is summarized in Scheme 1.



Scheme 1. Synthetic scheme of multifaceted thermoresponsive poly(N-vinylcaprolactam) coupled with carbon dots.

2.5. Characterization

IR spectrum was recorded using Shimadzu FT IR-8400S with a scanning range of 4000–400 cm^2. Transmission electron microscopy (TEM) studies were performed using JEOL JEM-2200FS at a voltage of 200 kV. The molecular weight of the as-synthesized polymer was measured using gel permeation chromatography (GPC) in H_2O (Breeze Systems, USA). The ^1H NMR spectrum was recorded in D2O with a 400 MHz Bruker spectrometer. Dynamic light scattering (DLS) measurements were performed using a Malvern Zetasizer Nano-ZS90 with a laser excitation wavelength of 633 nm and all presented data are an average of three measurements. Atomic force microscopy (AFM, NanoScope III, England) for a sample prepared by dropping the 0.01 mg/mL PVCL–CDs aqueous solution onto a plasma-treated Si wafer and drying under ambient conditions was performed in tapping mode. The UV–Vis and photoluminescence (PL) spectrum were recorded on a Jasco V–650 spectrophotometer and Jasco FP–6500 spectrofluorometer, respectively. The photobleaching measurement was performed with a Jasco FP–6500 spectrofluorometer using the built-in light source. Fluorescence images excited at 365 and 450 nm were recorded with a digital camera (Eclipse 80i, Nikon, Japan).

2.6. Cell studies

The NIH-3T3 (mouse embryonic) fibroblast cell line obtained from the American Type Culture Collection (ATCC) was cultured in DMEM supplemented with 10% FBS at 37 °C under 5% CO2 and 95% relative humidity. The cells (1 × 105) were seeded into a 4-well plate and incubated for 24 h. Then, 100 μL of a fresh aqueous solution containing PVCL–CDs (10 or 100 mg/L) was added. After 1, 3, or 5 days, MTT (100 μL) was added to each well and incubated at 37 °C for 4 h. All media were removed and then 150 μL of DMSO was added and kept for 15 min in the dark. The absorbance was measured at 570 nm using a kinetic microplate reader (EL 9800, BioTek Instruments Inc., Highland Park, USA) with pure DMSO as a blank. A non-treated cell was used as a control and the percent cell viability (n = 4) was calculated by Atest / Acontrol × 100, where Atest and Acontrol are the absorbances of the wells (with the PVCL–CDs) and control (without the PVCL–CDs), respectively. To examine temperature sensing and bioimaging applications, NIH-3T3 was seeded on coverslips in a 4-well chamber for 24 h and then cells were washed 3 times with water and imaged under a fluorescence microscope (Eclipse 80i, Nikon, Japan).

The temperature of the cell chamber was controlled using the heating glass of a live cell instrument (CU-201, South Korea), while the actual temperature of the cell chamber was calibrated using an infrared thermometer (Fluke 62 Max, USA). The fluorescence intensity was measured from digital images using ImageJ©.

3. Results and discussion

3.1. Synthesis of PVCL–CDs

A two-step synthetic protocol was employed to synthesize the fluorescent thermoresponsive PVCL–CDs, as shown in Scheme 1. In the first step, PVCL-COOH was synthesized by free radical polymerization using the water soluble initiator VA-057, which added COOH groups to the chain ends. The ^1H NMR spectrum of PVCL-COOH showed peaks at 1.4–1.7, 2.4, 2.8, and 3.6 ppm [20], which correspond to the characteristic protons of PVCL-COOH labelled in (Fig. S2). The peaks at ~4.7 ppm correspond to D2O. The absence of peaks at 7.5 ppm from the vinyl protons of the monomer also confirmed that the polymerization completely consumed the vinyl monomers [19]. The weight-average molecular weight (M_w) of the as-synthesized PVCL-COOH was 2.1 × 10^4 g/mol with a polydispersity (M_w/M_n, PDI) of 1.48. In the second step, the amine end groups of the synthesized CDs were reacted with the carboxylic groups of PVCL-COOH through EDC amidation [18]. FT-IR spectrum of CDs shows the intensity of the signal from the N–H stretching bond frequency of amide groups (Fig. S1).

UV–Vis spectroscopy was performed to confirm the successful synthesis of PVCL–CDs (Fig. 1). As shown in Fig. 1, the aqueous PVCL-COOH solution had no obvious absorption peaks because it contains no visible or near-UV chromophores, whereas the aqueous solution of free CDs showed a classical absorption peak at 260 nm due to a π–π* transition of C = C bonds and the absorption at 340 nm corresponds to a n–π* transition of C = O bonds [21]. The aqueous PVCL–CDs solution showed a π–π* transition of the coupled CDs at 344 nm, which was redshifted by 4 nm with a decreased intensity compared with that of the aqueous solution of free CDs. The redshift is due to the narrowed band gap that occurs upon amide bond formation between PVCL-COOH and the CDs, which has a noticeable contribution to the electronic structure of PVCL–CDs [20].

The particle sizes of 1 wt.% PVCL-COOH and PVCL–CDs aqueous solutions were measured by DLS as a function of temperature (Fig. 2). The Z-average sizes of PVCL-COOH and PVCL–CDs (108 and 143 nm, respectively) increased noticeably at ~33 °C (714.857 and 848.57 nm, respectively). These increases occurred at their LCSTs due to a change from inter-chain hydrogen-bonding (between the amide group of PVCL and water) to hydrophobic interactions (which gradually leads to dehydration). The LCST of PVCL is known to vary from 30 to 50 °C depending on the molecular weight, thus the measurement of the LCST of PVCL indirectly reveals its molecular weight [20]. The LCST transition at 33 °C indicates a molecular weight of ~27,000 g/mol, which is in good agreement with the GPC data.

Fig. S3 shows the AFM image of the PVCL–CDs with the corresponding thickness profile. The AFM sample was prepared by dropping a 0.005 mg/mL PVCL–CDs aqueous solution onto a plasma-treated Si wafer and drying under ambient conditions. The PVCL–CDs particles are well separated with an average diameter of ~40 nm and thickness of 2.5 nm. The increased diameter confirmed the successful incorporation of PVCL with the CDs, as the size of a CDs is usually less than 10 nm [18]. The TEM images of CDs (Fig. S4a) and PVCL–CDs (Fig. S4b-c) show that CDs have diameters of 3.2 to 4.5 nm. The CDs have a crystalline structure consisting of parallel crystal planes with lattice spacing of 0.23 nm, matching the d-spacings of (100) and (002) planes of graphite [22].

From a fundamental and application-based view, photoluminescence (PL) is one of the most fascinating features of PVCL–CDs. Fig. 3a shows the excitation and emission spectrum of a 10 mg/mL

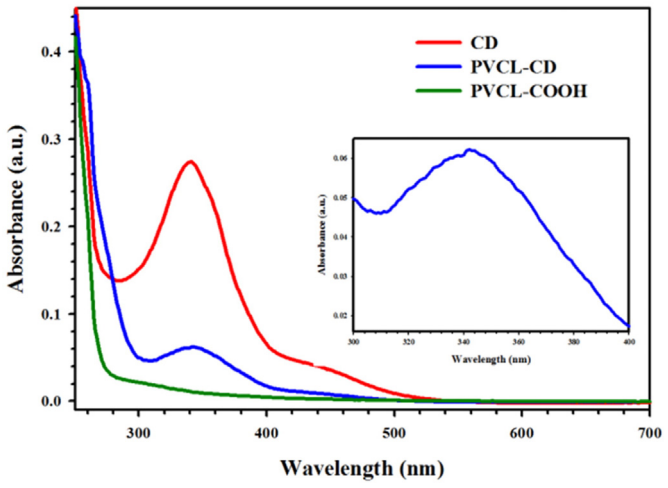


Fig. 1. UV–Vis spectrum of aqueous solutions of PVCL-COOH (0.1 wt.%), CDs (1 mg/mL), and PVCL–CDs (1.1 wt.%, which corresponds to 1 mg/mL of coupled CDs, determined by subtracting the amount of unreacted CDs after EDC coupling from the initial amount of CDs at 26 °C.

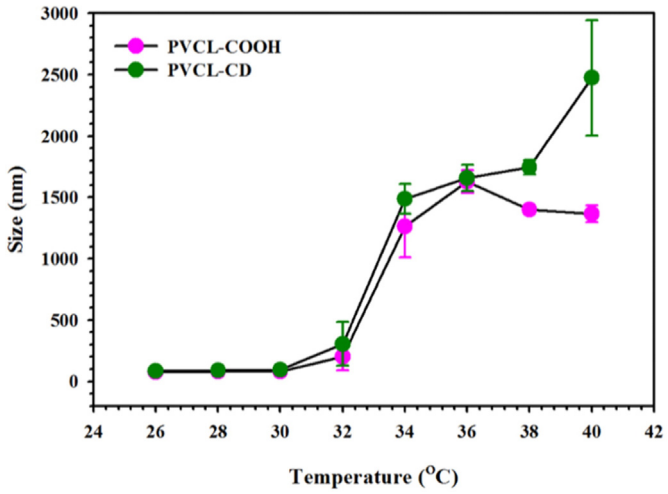


Fig. 2. DLS measurements of 1 wt.% PVCL-COOH and PVCL–CDs aqueous solutions at various temperatures. The presented data is an average of three measurements.

PVCL–CDs aqueous solution at 25 and 45 °C, while the inset photographs show the visible effect when irradiated at 360 nm. At 25 °C, the aqueous PVCL–CDs solution shows an excitation maximum at 360 nm. When irradiated at 360 nm, blue fluorescent light at 437 nm was observed through the transparent solution. This fluorescence results from the radiative recombination of excitations, produced by the CDs, at surface energy traps [5]. When the temperature increased above the LCST at 45 °C, the solution became turbid with a noticeable decrease in luminescence because of the collapse of PVCL chains caused by the temperature-induced hydrophobicity. It is noteworthy that the fluorescence emission intensity decreased to about 36% when the temperature rose from 25 to 45 °C. The thermally induced aggregation of PVCL above LCST would embed part of fluorescent CDs inside hydrophobic core thus decreasing the fluorescent intensity [17,23,24].

Investigation of the temperature-dependent fluorescence of PVCL–CDs was carried out in terms of the PL response to temperature to determine the applicability of the material in sensor and intracellular thermometer applications. Fig. 3b shows the fluorescence intensity of the 10 mg/mL PVCL–CDs aqueous solution as a function of temperature. When excited at 360 nm, the PL emission intensity at 437 nm decreased in a stepwise manner, starting at 33 °C, as the temperature increased, which is similar to the results of the DLS measurements in Fig. 2. Thus, the fluorescence decrease in Fig. 3a closely correlates with the LCST and is caused by the collapsed chain structure above the LCST. Fig. 3c shows the change in the PL intensity of the 10 mg/mL PVCL–CDs aqueous solution during heating/cooling cycles between 25 and 45 °C. The system exhibits good reversibility of the temperature-dependent fluorescence decrement/enhancement during the heating/cooling cycles. The reversibility of the fluorescence response to temperature makes this material useful as a CD-based thermo-sensitive sensor.

An important advantage of CDs over semiconductor QDs and organic fluorophore is their resistance to photobleaching, which can be determined by the photostability of the aqueous PVCL–CDs solution.

Fig. S5 shows the photostability of the 10 mg/mL PVCL–CDs aqueous solution as a function of time at 25 and 45 °C at an UV power of 2 mW/cm^2. After an exposure of 500 s, the total fluorescence emission decreased by ~3 and 8% at 45 and 25 °C, respectively. These small decreases indicate that the CDs are resistant to photobleaching. The increased photostability

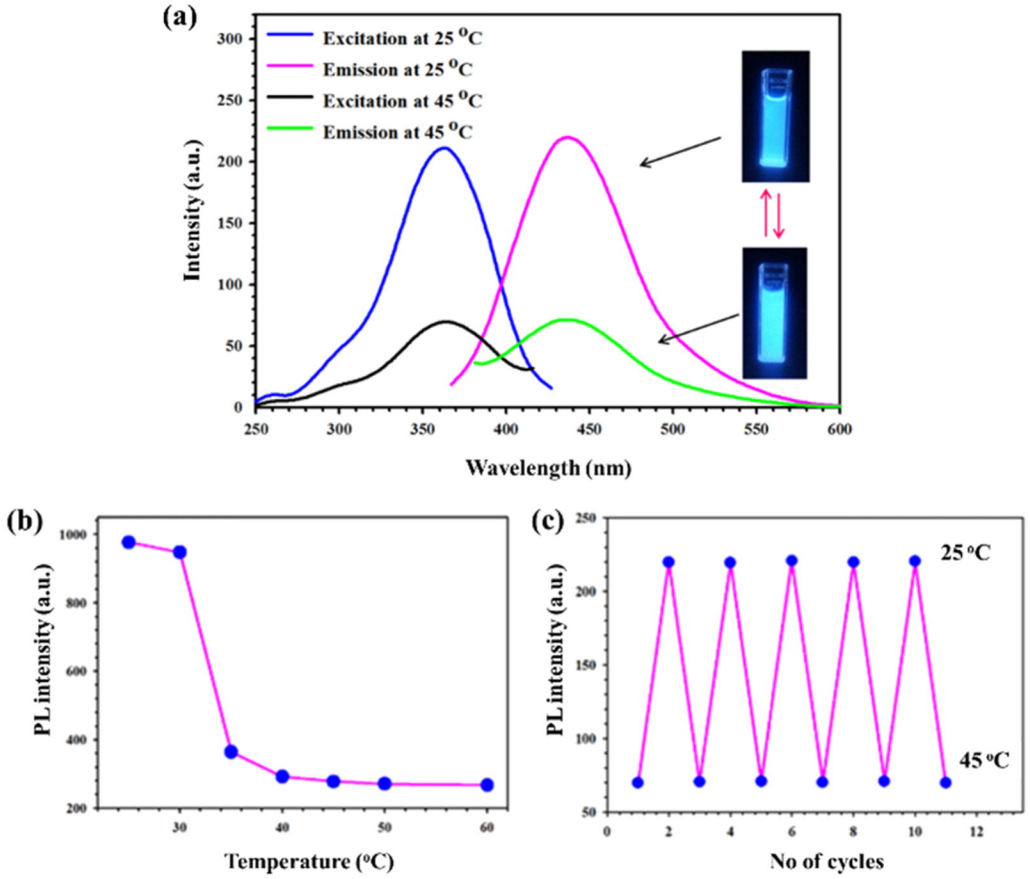


Fig. 3. (a) Excitation and emission spectrum of the 10 mg/mL PVCL–CDs solution at 25 and 45 °C. The inset photographs show the solution after irradiation at 360 nm. (b) The fluorescence intensity at 437 nm for the 10 mg/mL PVCL–CDs aqueous solution as a function of temperature when excited at 360 nm. (c) The change in PL intensities of the 10 mg/mL PVCL–CDs aqueous solution during heating/cooling cycles between 25 and 45 °C. (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

(reduced photobleaching) of PVCL–CDs at 45 °C may be due to the thermally induced collapse of PVCL above its LCST, which encloses the CDs inside the PVCL chains and protects them from light. The system also shows no photoblinking at either temperature for the given exposure time, which is an advantage of PVCL–CDs because photoblinking is usually observed in QD solutions [25]. The water-solubility, remarkably high photostability, and no photoblinking suggest that PVCL–CDs could perform well in biomedical applications under long exposure times.

3.2. Protein sensor

Proteins can be adsorbed on PVCL–CDs by several mechanisms, such as hydrophobic interactions, hydrogen bonding, and electrostatic interactions [26,27].

The adsorption of proteins would change the PL intensity. If the PL intensity of the aqueous PVCL–CDs solution increases with protein addition, the system has potential application as a biosensor. Fig. 4 shows the PL intensities of the aqueous PVCL–CDs solution (10 mg/mL) at 437 nm as a function of concentration of added BSA and lysozyme in a neutral pH of 7 at 25 and 45 °C when excited at 360 nm. As shown in Fig. S6, the fluorescence emission intensities of the aqueous PVCL–CDs solution at 45 °C (above the LCST) substantially increase upon the addition of BSA (or lysozyme) until a concentration of ~350 nM was reached. At higher protein concentrations, the intensities were saturated with concurrent bathochromic (red) shifts of the maximum peak. The increase in PL intensity above the LCST with an increase in protein concentration indicates that the aqueous PVCL–CDs solution can be used as a nonspecific biosensor.

The addition of small molecules like salt, protein or any other organic additives like glucose or starch can alter polymer–water interactions and thus tune the LCST of the thermo-sensitive polymer [28]. The proposed reason for such tuning is that the addition of electrolytes changes the water structure and the free energy of interaction between polymer and water which modifies the water molecule orientation in the hydration shell surrounding polymer which increases the hydrophobicity of the polymer and this results in a reduction in its LCST [27]. In this context, in our case the added proteins act as an electrolyte causing a reduction in PVCL–CDs LCST and the solution became more transparent with the addition of protein thus increasing the fluorescence intensity up to the concentration of 350 nM. This turn-on response of the PVCL–CDs to added proteins indicates their possible use as quantitative biosensors.

3.3. Cell studies

Biocompatibility is important for the application of as-prepared PVCL–CDs to intracellular thermometers and fluorescent probes in cell imaging. Fig. 5 shows the MTT assay for cell viability of NIH 3T3 cells treated with 0 (control), 10, and 100 mg/L PVCL–CDs for 1, 3, and 5 days. Over 85% of cells survived for up to 5 days in samples treated with 10 and 100 mg/L PVCL–CDs solutions, indicating that PVCL–CDs is remarkably biocompatible with NIH 3T3 cells. Notably, the concentrations used in the in-vitro assessment are much higher than those used in potential applications (20 μg/mL), such as optical imaging of live cells [29]. Thus, the exceptional biocompatibility of PVCL–CDs makes the system useful in novel biological applications.

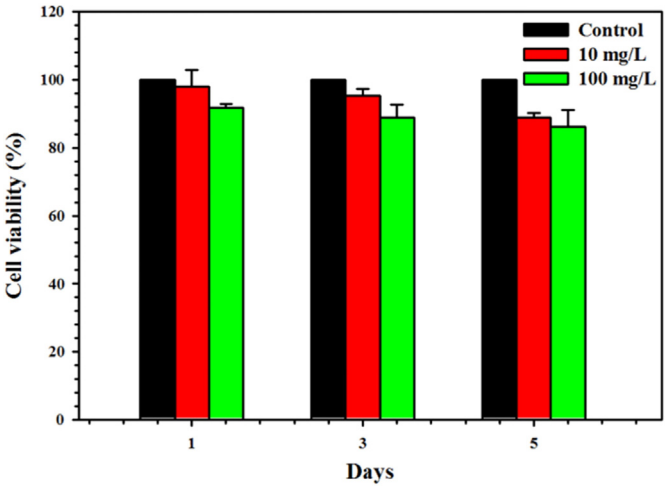


Fig. 5. MTT assay for cell viability of NIH 3T3 cells treated with 0 (control), 10, and 100 mg/L PVCL–CDs for 1, 3, and 5 days.

Imaging of intracellular temperatures is an important method for tracking cellular events [30]. For example, cancer cells can be distinguished from normal cells by imaging the intracellular temperature because the temperature of cancer cells is higher than that of normal cells [23]. Fig. 6a, b shows fluorescence microscopy images of NIH 3T3 cells incubated in 1 wt.% aqueous PVCL–CDs solution for 24 h when excited at 365 nm. Most of the PVCL–CDs was uptaken by the cells and blue fluorescence was observed for all cells imaged at different temperatures. The brightness of the images was calculated from the grey-scale intensity. Fig. 6c shows the grey-scale intensity as a function of temperature, which clearly decreases as the temperature increases. This result indicates that fluorescence quenching can occur in the NIH 3T3 cells, demonstrating that the biocompatible PVCL–CDs could be used as an intracellular thermometer for novel diagnosis of diseases.

The PL spectrum of the 10 mg/mL PVCL–CDs aqueous solutions excited at different UV wavelengths are shown in Fig. S7. The wavelength of the PL peak of the CD solution was known to be strongly dependent

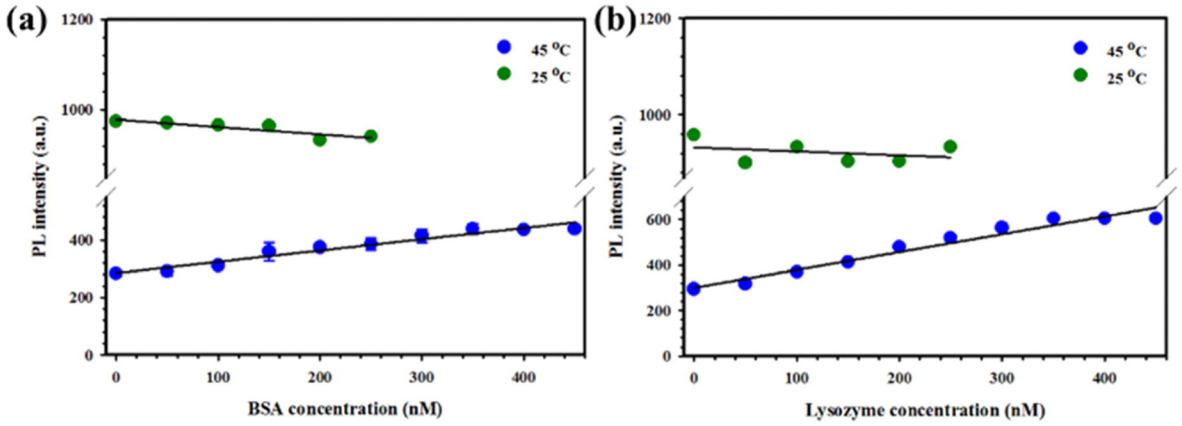


Fig. 4. PL intensities of the aqueous PVCL–CDs solution (10 mg/mL) at 437 nm as a function of concentration of added (a) BSA or (b) lysozyme in a neutral pH of 7 at 45 and 25 °C when excited at 360 nm.

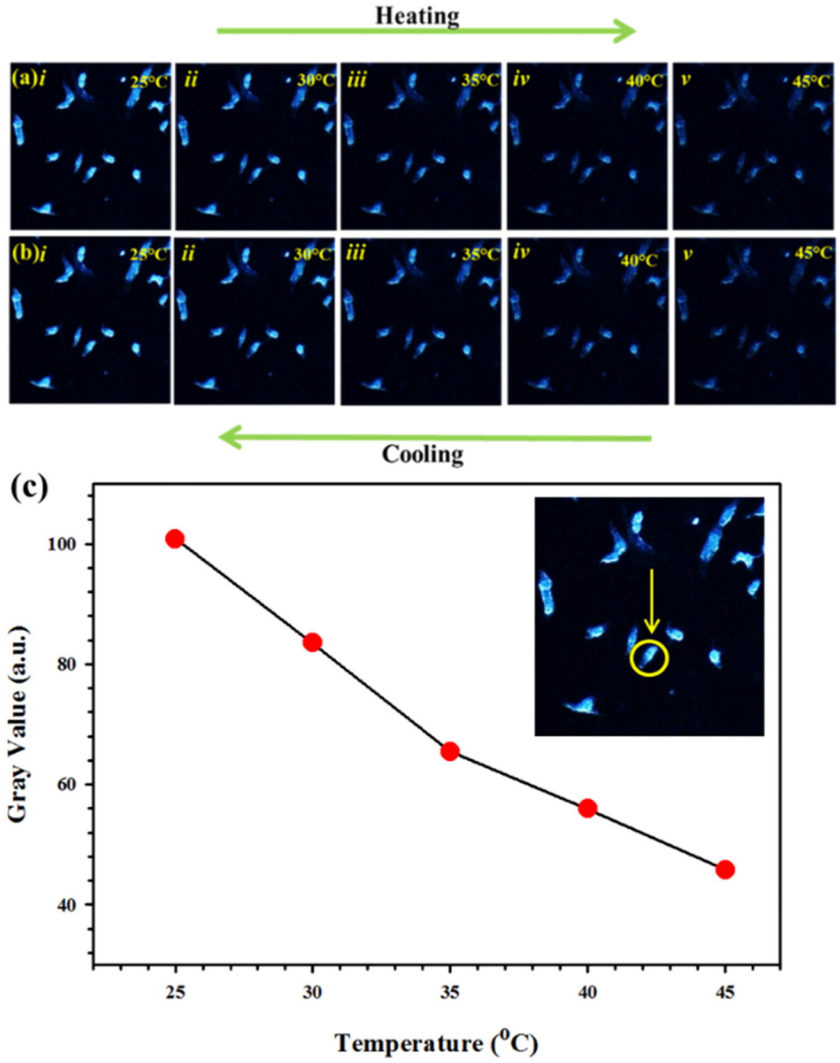


Fig. 6. Fluorescence microscopy images of the NIH 3T3 cells incubated with the 10 mg/mL PVCL–CDs aqueous solution during (a) heating (→) and (b) cooling (←) at (i) 25, (ii) 30, (iii) 35, (iv) 40, and (v) 45 oC. (c) Grey-scale intensity of the images of the circled area in inset (Fig. 6a) during heating as a function of temperature. (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

on the wavelength of the exciting UV light [24]. The same excitation-dependent emission of PVCL–CDs was observed in the PVCL–CDs solution. This excitation-dependent emission of PVCL–CDs is an advantage of CDs over other labelling agents due to its ability to show various colours during bio-imaging by controlling the exciting UV wavelength. We tested the change in fluorescence colour of PVCL–CDs in the NIH 3T3 cells in white light and by controlling the UV wavelength (Fig. S8). The blue colour emitted by excitation at 365 nm changed to green when the excitation wavelength was changed to 450 nm. This colour change demonstrates that PVCL–CDs can be used as a tracer in bioimaging and serves as a substitute for organic dyes and semiconductor QDs due to its biocompatibility and versatility.

4. Conclusions

A thermoresponsive fluorescent material, PVCL–CDs, was synthesized via free radical polymerization and EDC coupling. The aqueous PVCL–CDs solution exhibited excellent biocompatibility and an LCST of 33 °C (at a weight-average molecular weight of 2.1×10^4 g/mol). At temperatures above the LCST, the fluorescence intensity notably decreased due to the thermally induced collapse of PVCL chains. This multifunctional material was applied as a quantitative protein biosensor, intracellular temperature sensor, and bioimaging marker. PVCL–CDs exhibited a turn-on fluorescence response above the LCST upon injection of a protein (BSA or lysozyme) solution with concentrations up to 350 nM. PVCL–CDs was easily absorbed by NIH 3T3 cells and acted as a fluorescence probe in bioimaging. The fluorescence intensity of PVCL–CDs was dependent on the cell temperature and the colour of the PVCL–CDs fluorescence could be tuned by the UV excitation wavelength. Hence, we believe this multifaceted PVCL–CDs material could be a useful analytical tool for protein assays, cancer targeting via temperature imaging, bioimaging, and many other biomedical and clinical applications.

Acknowledgements

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Appendix A. Supplementary data

Detail experimental data of elemental analysis; FT-IR spectrum; ^{1}H, NMR spectrum; TEM images; Fluorescence emission spectrum; PL spectrum; and the fluorescence microscope images. Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.msec.2015.12.070.

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